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## CLIMATIC EFFECTS ON FORAGING PERFORMANCE OF BEEF COWS ON WINTER RANGE

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*Range Experiment Station, Manyberries, Alberta*

[Received for publication January 5, 1954]

### ABSTRACT

A study was made of the effects of climatic factors on foraging performance of beef cows at the Range Experiment Station, Manyberries, Alberta. The relationships among (1) percentage of cows foraging daily on upland open winter range; (2) daily mean temperature in degrees F.; (3) daily wind mileage, and (4) amount of supplemental feed given, were determined. A feeding index with a range of 1 to 4 was used, 1 indicating no supplemental feed given and 4 indicating 1½ lb. of linseed oilcake and 15-20 lb. roughage per head per day. Data were obtained on domestic cows of the Hereford, Shorthorn, and Angus breeds, and Cattalo cows of half-bison and quarter-bison breeding, during January, February, and March, 1952.

Covariance analysis revealed no significant difference between breeds in the degree of correlation for the characteristics studied. The combined gross correlation coefficients  $r_{12}$ ,  $r_{13}$ , and  $r_{14}$  were 0.416\*\*, -0.169\*\*, and -0.162\*\*. The partial correlation coefficients  $r_{12.34}$ ,  $r_{13.24}$ , and  $r_{14.23}$  were 0.382\*\*, -0.088, and -0.086, and the corresponding regression coefficients were 0.778, -0.036, and -3.322. The regression was linear. The multiple correlation coefficient  $r_{1.234}$  at 0.432 was not much higher than the coefficient  $r_{12.34}$  at 0.382, indicating that temperature was the most important environmental factor studied.

The Cattalo foraged on open range more frequently than the cows of domestic breeds. Also, a greater proportion of Cattalo cows were out foraging as compared with cows of domestic breeds.

\*\*Significant at the 0.01 level.

### INTRODUCTION

Winter grazing by range beef cattle reduces winter feeding costs. It is important, therefore, to have cattle that will graze under unfavourable weather conditions. Little specific information is available regarding differences between breeds or strains in their willingness or ability to forage under extreme winter conditions. Experience has shown that Canadian domestic breeds are subject to heavy death losses during periods of heavy snow cover and extreme cold unless feed, in the form of roughage or supplements, is provided. Certain wild species apparently are able to survive such conditions, though comparative data on their behaviour are meagre or non-existent.

An opportunity to study differences between breeds of domestic cattle and bison-cattle hybrids exists at the Canada Range Experiment Station, Manyberries, Alberta. This paper presents the results of studies of grazing habits during the winter of 1951-52.

<sup>1</sup> Range Management Assistant and Superintendent, respectively.



### MATERIALS AND METHODS

The breeding cows at the Manyberries Station are wintered on native rangeland typical of the shortgrass prairie. A deep coulee, known as Lost River, crosses the field diagonally, providing shelter from the wind. Native hay, cereal straw, and grain or protein supplement (commonly linseed oilcake), are placed in the winter field for emergency use and are fed when conditions make it difficult or impossible for animals to graze. In some winters very little feeding is necessary. On the other hand, feeding began early in January and continued until April in the winter of 1951-52, when the range was still covered with several inches of snow and cold weather prevailed. Mean monthly temperatures for that winter, with the 23-year means in brackets, were as follows: December, 8.3° F. (16.3° F.); January, 3.2° F. (10.2° F.); February, 17.6° F. (12.6° F.); and March, 15.5° F. (24.6° F.).

A daily record, taken in mid-day, was kept of the number of animals found grazing on upland range during a 79-day period (January 6 to April 1, 1952, excluding 8 days in February, when the herd was moved to the Station buildings for collection of hair samples for fineness and density determinations). The Angus and Shorthorn cows used in the study were 3 years of age; the Herefords ranged from 3 to 11 years; the quarter-bison Cattalo 3 to 12 years, and the half-bison Cattalo 13 to 18 years of age.

Mean daily temperature was derived from minimum and maximum temperature readings recorded on typical upland at headquarters, approximately 3 miles distant from the winter range. The area is quite level, with no brush to impede wind movement. The wind mileage per day was recorded at headquarters also. Hourly and daily wind mileage were recorded by anemograph. An index was derived to account for the amount of feed given on each day when some feed was supplied to the range herd. A feed index of "1" represents no supplemental feed given; "2" indicates an allowance of 1½ lb. of linseed oilcake per head; "3" indicates an allowance of 15-20 lb. of roughage per head (approximately equal amounts of non-legume hay and wheat straw); and "4" indicates that both linseed oilcake and roughage were fed in the amounts shown under "2" and "3", respectively. Some feed was provided on 59 days of the 79-day experimental period because of deep snow and/or cold weather.

A study was made of the relationship between percentage of animals foraging daily (rustling) and the daily mean temperature, wind mileage, and amount of supplemental feed. Gross, partial, and multiple correlation coefficients were calculated and the rectilinearity of regression was tested by the methods of Treloar (2 and 3). Covariance analysis was used to test the heterogeneity of regression between breeds before combining the the gross correlation coefficients for the different classes of cattle by the *r* to *z* transformation of Fisher (1).

### EXPERIMENTAL RESULTS

Table 1 gives a summary of the foraging performance of the cows of the different breeds during the 79-day period. The foraging refers to actual grazing on upland except for four days in March when the half-bison cows grazed in the draws where feed was more readily available. During these four days the domestic cattle stayed near the feed-ground.



TABLE 1.—FORAGING PERFORMANCE OF COWS ON WINTER RANGE AT MANYBERRIES, 1952

	Hereford	Shorthorn	Angus	Quarter-bison Cattalo	Half-bison Cattalo
Number of animals	93	13	10	26	39
Number of days on which some or all of the animals in each group grazed	34	25	26	41	50
Average per cent of animals out grazing when some grazed	49.5	42.1	71.5	61.1	96.2
Average per cent of animals out grazing (79-day period)	21.3	13.3	23.5	31.7	60.9

TABLE 2.—PERCENTAGE OF ANIMALS GRAZING DAILY (1), AS RELATED TO (2) MEAN ENVIRONMENTAL TEMPERATURE, (3) DAILY WIND MILAGE, AND (4) AMOUNT OF SUPPLEMENTAL FEEDING

Breed	$r_{12}$	$r_{13}$	$r_{14}$
Hereford	0.294**	-0.072	-0.171
Shorthorn	0.320**	-0.110	-0.189
Angus	0.296**	-0.108	-0.163
Quarter-bison Cattalo	0.649**	-0.246*	-0.221
Half-bison Cattalo	0.464**	-0.303**	-0.066

\*\* P. = 0.01.

\* P. = 0.05.

The data in Table 1 indicate that the Cattalo grazed during a greater number of days than the domestic cattle. Likewise, a greater proportion of all of the Cattalo went out to graze on grazing days than was the case with the domestic cattle. On all but five days, the 39 half-bison cows (first-cross hybrids) were together as a group, whether on upland range or in the coulee. They were particularly willing and able to graze under unfavourable conditions. They did not paw to uncover the grass, as horses do, but burrowed with their muzzles through the snow to secure feed.

Table 2 gives the correlation coefficients for percentage of animals of each breed out grazing daily and mean temperature, wind milage, and feed index. Mean temperature for the 79-day period was 13.1° F.; the average wind milage per day was 274, and the average feed index was 2.67.

Covariance analysis revealed no significant difference between breeds in the degree of correlation for the characteristics studied. Table 3 combines the correlation data and gives the results of partial and multiple correlation analysis.

The second-order partial correlation coefficient between percentage of animals grazing per day and the mean temperature ( $r_{12.34}$ ) was highly significant. The gross correlation coefficients  $r_{13}$  and  $r_{14}$  were significant,

TABLE 3.—SUMMARY OF CORRELATION AND PARTIAL CORRELATION ANALYSES OF FORAGING DATA

r	2	3	4	23	24	34	234
1	0.416**	-0.169**	-0.162**				0.432**
2		-0.200	-0.186			0.102	
3			0.135		-0.164		
12		0.396**				0.382**	
13	-0.096				-0.088		
14	-0.095	-0.142		-0.086			

\*\* P. = 0.01.

\* P. = 0.05.

but the partial correlation analysis, which removed the temperature effect, reduced these coefficients to non-significant levels. Thus the mean temperature was the most important environmental factor studied in so far as the relationship to the amount of foraging by animals was concerned. In fact, the coefficient  $r_{12.34}$  at 0.382 is not greatly lower than the multiple coefficient  $r_{1.234}$  at 0.432. A test of the correlation ratio, using Woo's table (4), showed this to be a linear relationship.

The partial regression coefficients  $b_{12.34}$ ,  $b_{13.24}$  and  $b_{14.23}$ , were 0.778, -0.036, and -3.322, respectively. Although the regression coefficient  $b_{12.34}$  is statistically significant, the correlation is not high enough to warrant further regression analysis.

The average winter weight losses of cows on the experiment ranged from 78 lb. for the half-bison hybrids to 159 lb. for the Shorthorns. More data are required to compare the different types of cattle in weight maintenance on winter range.

#### ACKNOWLEDGEMENT

The data on foraging of cattle and amount of supplemental feed given were collected by R. E. Foster, Cattle Herdsman at the Station.

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# WATER DISTRIBUTION PATTERNS FROM ROTARY SPRINKLERS<sup>1</sup>

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[Received for publication November 1, 1954]

## ABSTRACT

Factors affecting the uniformity of distribution of water by rotating sprinklers were studied. Eight geometric curves of water distribution by sprinklers were selected. In addition, examples of such curves were selected from field tests for study. In all cases, various sprinkler spacings were assumed, both square and rectangular; the distribution of water between sprinklers was mapped; and the degree of uniformity of distribution within the areas studied was calculated. Conclusions reached were as follows:

1. The best type of distribution curve for general application is one showing a steady decrease in rate of water application from the sprinkler out toward the outer circumference of water throw.
2. The attainment of this type of curve does not of itself guarantee good water distribution. The uniformity coefficient and the range coefficient are suggested for evaluating the uniformity of distribution.
3. With square spacing of the sprinklers, the spacing should be not greater than 60 per cent of the diameter of the throw.
4. On the average, square spacing of the sprinklers gives more uniform water distribution than does rectangular spacing covering the same area. This applies only to a condition of little or no wind.

## PURPOSE OF INVESTIGATION

Sprinkler irrigation has been found (10) to provide more uniform distribution of water in the soil than do other methods of irrigation. Even with sprinkler irrigation, however, the distribution of water is often far from uniform. Such a lack of uniformity minimizes what should be considered one of the major advantages of the sprinkler method. It is highly desirable, therefore, that everything possible be done to assure good uniformity of distribution by sprinklers. In order to accomplish this it is necessary, first, to know how to measure this uniformity, and second, to understand some of the factors affecting it.

A general procedure for determining the efficiency of water distribution by individual sprinklers was reported in 1947 by Wilcox and Swailes (9). This procedure followed closely previous work reported by Christiansen (3, 4). It consisted of the field operation of a single sprinkler, measurement of the water in cans spaced around the sprinkler in a grid pattern, overlapping of the distribution patterns on paper diagrams for different assumed sprinkler spacings, and calculation of coefficients of uniformity for each spacing. The method has given valuable results. The last two steps in the procedure, however, have proved to be somewhat tedious. The question has arisen as to whether, with the background already established, it might be possible to assess the uniformity of distribution by a somewhat simpler procedure; whether it would be possible, for example, to use the curve of water distribution from the sprinkler nozzle outwards for this purpose.

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<sup>3</sup> Technician, plant nutrition, soils and irrigation investigations.

This paper presents the results of studies instituted to determine whether there is any relationship between the type of water distribution curve and the uniformity of water distribution when a sprinkler spray overlaps; and if so, whether there is any ideal type of curve associated with high uniformity of distribution. As a by-product of these studies, data are also presented dealing with desirable sprinkler spacings.

### REVIEW OF LITERATURE

Many workers have tested the distribution of water from sprinklers. Comparatively few, however, have reported the use of curves of distribution as an indication of efficiency of distribution. In 1931, Staebner (8) reported a number of tests of spray distribution. From 1937 to 1942, Christiansen (2, 3, 4) reported comprehensive tests of water distribution. The procedures used for testing distribution in the field and calculating the coefficients of uniformity were outlined in detail. The water was caught in cans spaced in a grid pattern around individual sprinklers. Overlapping was mapped on paper diagrams for different sprinkler spacings. The uniformity coefficient ( $C_u$ ) was calculated for overlapping sprays as follows:

$$C_u = 100 \left( 1 - \frac{Sd}{mn} \right)$$

in which  $Sd$  = the sum of the deviations from the mean value of the depths of water caught in the cans,  $m$  = the mean value and  $n$  = the number of observations. Six different theoretical types of distribution pattern were selected for study (3, 4), and uniformity coefficients were calculated for each type assuming different sprinkler spacings. For reasonably close spacings between the sprinklers, a distribution pattern showing a steady decrease in rate of application from the sprinkler outward produced the highest uniformity coefficients. Assuming the same distribution at all points of the compass around the sprinkler, the greatest square spacing between sprinklers that gave good distribution was 71 per cent of the wetted diameter.

Most of the work reported in the past 10 years has been done using Christiansen's procedures. In 1946, McCulloch and Bowman (7) calculated uniformity coefficients using the same formula, and depicted some of the distribution curves obtained. In 1947, Wilcox and Swailes (9) used a modified procedure for determining the uniformity coefficient, as follows:

$$U = 100 - \frac{100 SD}{M}$$

in which  $U$  = uniformity coefficient,  $SD$  = standard deviation of depths of water in cans,  $M$  = mean of depths of water. This equation lays special stress on the extreme values. A number of typical distribution curves were also presented. It was suggested that a uniformity coefficient of at least 70 is desirable. In 1948, Kemp, Halliday and Spurling (5) published a number of distribution curves from sprinklers of different types. They did not, however, state the degree of uniformity of water distribution over the area covered. In 1949, the United States Soil Conservation Service (1) suggested a distribution curve with a steadily decreasing rate of water application from the sprinkler outward as being the most satisfactory



type. In 1952, Korven (6) used uniformity coefficients as a measure of uniformity of distribution between sprinklers placed in a rectangular pattern in the field.

### PROCEDURE

#### *Theoretical Curves*

Eight different types of distribution curve were selected for study, as depicted in Figure 1. Most of these curves were of types normally encountered in actual irrigation tests; some were included for their academic interest only. Some were similar to the geometric curves previously

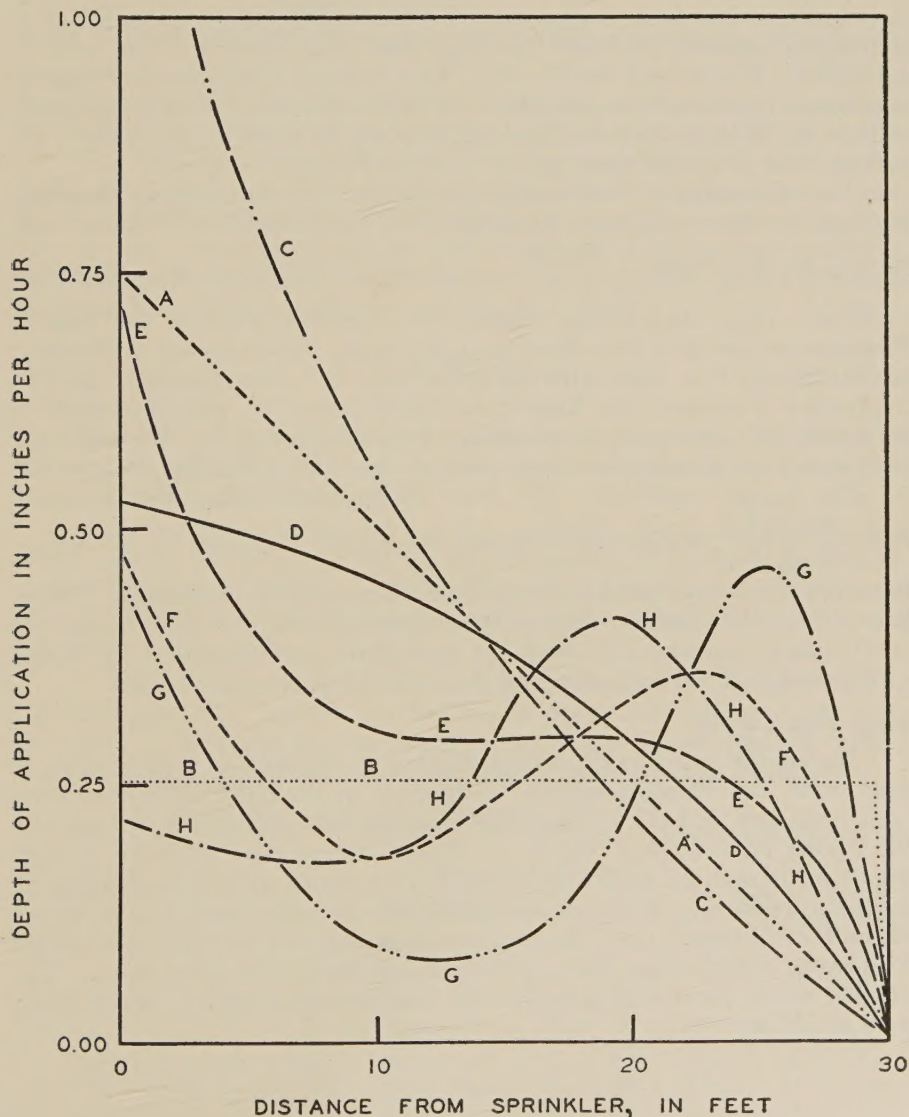


FIGURE 1. Eight sprinkler distribution patterns used for special study.

studied by Christiansen (3). Each curve represents a radius of throw of the sprinkler of 30 feet. The position of each curve is so arranged that the total amount of water delivered by the sprinkler is the same for all curves. As far as possible, all curves (or part curves where their direction changes) in Figure 1 represent part circles.

It was assumed that each of the theoretical distributions illustrated in Figure 1 was obtained by setting the sprinkler in the centre of an area in which open cans were placed in a  $5 \times 5$  foot square pattern. The depth of water caught in each can would depend on the curve under consideration and the distance from the sprinkler. When the water distribution around each sprinkler had been mapped in this manner, the pattern for each of a number of assumed sprinkler spacings was determined by the method outlined by Wilcox and Swailes (9). This method allows for overlapping of patterns from adjacent sprinklers. Calculations were made for spacings of  $20 \times 20$ ,  $20 \times 30$ ,  $20 \times 40$ ,  $20 \times 50$ ,  $20 \times 60$ ,  $25 \times 25$ ,  $30 \times 30$ ,  $40 \times 40$  and  $50 \times 50$  feet with each type of curve in Figure 1.

The uniformity of distribution of water for each sprinkler spacing was assessed by three different methods: (a) The formula of Wilcox and

Swailes (9),  $U = 100 - \frac{100 \text{ SD}}{M}$ , was used to determine the uniformity

coefficient (U). As already noted, this equation stresses the widely divergent values more than those near the mean. With perfect uniformity of distribution,  $U = 100$ ; with less uniformity,  $U = \text{less than } 100$ . (b) For comparison purposes, the formula of Christiansen (3) was also used to determine the uniformity coefficient. Values obtained by this equation were somewhat higher than those obtained by that of Wilcox and Swailes. (c) The range coefficient (R) was determined, using the formula

$R = \frac{200 (H-L)}{H + L}$ , in which H = highest value and L = lowest value. When

$R = 200$ , the lowest value is zero and the range is at its maximum. When  $R = 0$ , there is perfect uniformity of distribution. An examination of many charts indicates that both the uniformity coefficient and the range coefficient are of value in assessing the uniformity of a distribution.

#### *Field Test Curves*

During the past 9 years, a large number of distribution tests have been made with individual sprinklers. A number of the charts obtained have been examined, and curves have been selected that approximate closely to the curves illustrated in Figure 1. These curves were obtained mostly from tests of orchard sprinklers with different nozzle sizes and operating at different pressures. Pressures too low for the sprinkler usually gave curves of the F or G type. Only those curves were selected that were associated with water distributions showing little if any skew effect from wind. The number of curves in each general class that were selected for special study were as follows:

- A - 15
- C - 13
- E - 18
- F - 15
- H - 15



In no case was the curve selected exactly the same as illustrated in Figure 1, though it was always similar in general type. Because it was so difficult to separate them, the G-type curves were combined with the F-type curves.

Studies were made on the water distribution chart associated with each curve. Uniformity coefficients (Wilcox and Swailes method) and range coefficients were calculated for spacings of  $20 \times 20$ ,  $25 \times 25$ ,  $30 \times 30$ ,  $40 \times 40$ ,  $50 \times 50$ ,  $20 \times 30$ ,  $20 \times 40$ , and in some cases  $20 \times 50$  and  $20 \times 60$  feet. The spacing coefficients (S) were also calculated, using the following

formula:  $S = \frac{100 \sqrt{\text{area included in spacing}}}{\text{diameter of throw}}$ . Thus if the diameter of

throw were 40 feet and the spacing  $20 \times 30$  feet, S would be  $\frac{100\sqrt{20 \times 30}}{40}$

= 61.2. By this formula, the same spacing coefficient was obtained irrespective of whether the spacing was square or rectangular, as long as the area represented was the same. With square spacing, the spacing coefficient could be defined as the sprinkler spacing expressed in per cent of the diameter of throw. Coefficients of correlation were calculated between the uniformity coefficient and the square of the spacing coefficient, and between the uniformity coefficient and the range coefficient. In almost all cases, the regressions showed straight-line trends.

## RESULTS

### *Theoretical Curves*

The uniformity coefficients obtained are summarized in Table 1, and the range coefficients in Table 2. An examination of these two tables reveals the following information:

1. As the area represented by the sprinkler spacing increased, there was a general tendency for the uniformity coefficient to decrease and the range coefficient to increase. This held true whether the area was increased by increasing the length of the one side only, or whether both sides were increased equally as in a square.

2. The trends accompanying increase in spacing were seldom uniform. Unexpectedly high uniformity coefficients sometimes occurred at wider spacings. In general, the less regular the pattern curve, the less regular were the trends of the uniformity coefficient and the range coefficient.

TABLE 1.—UNIFORMITY COEFFICIENTS FOR EIGHT CURVES

Type of curve	Sprinkler spacing in feet								
	20 X 20	20 X 30	20 X 40	20 X 50	20 X 60	25 X 25	30 X 30	40 X 40	50 X 50
A	96	91	82	57	36	95	86	75	40
B	90	84	79	85	68	89	93	65	62
C	93	91	71	47	27	92	90	60	23
D	94	90	90	66	43	92	73	62	51
E	86	84	85	79	55	92	86	80	66
F	83	78	71	81	59	88	68	61	58
G	70	81	55	62	61	72	58	41	29
H	84	73	64	80	55	80	64	54	53

TABLE 2.—RANGE COEFFICIENTS FOR EIGHT CURVES

Type of curve	Sprinkler spacing in feet								
	20 × 20	20 × 30	20 × 40	20 × 50	20 × 60	25 × 25	30 × 30	40 × 40	50 × 50
A	12	13	50	128	188	17	16	107	200
B	29	40	20	40	100	22	33	100	200
C	18	25	79	121	193	25	34	144	200
D	14	31	34	105	186	21	47	76	200
E	37	47	37	64	159	26	60	62	200
F	91	76	79	57	156	34	89	114	200
G	84	79	110	133	108	102	139	170	200
H	43	86	83	53	178	58	92	124	200

TABLE 3.—UNIFORMITY COEFFICIENT DATA FROM FIELD TESTS

	Type of spacing	Type of curve				
		A	C	E	F	H
Number of pairs correlated	Square	60	46	67	58	52
	Rectangle	42	39	52	52	44
Coefficient of correlation*	Square	-0.94	-0.86	-0.65	-0.83	-0.63
	Rectangle	-0.80	-0.88	-0.46	-0.68	-0.80
U when S = 50	Square	82	73	76	73	76
	Rectangle	75	65	76	69	78
U when S = 60	Square	74	61	71	66	71
	Rectangle	64	46	70	58	67
U when S = 70	Square	64	53	65	58	64
	Rectangle	50	24	62	45	53
S when U = 70	Square	64	52	62	54	63
	Rectangle	55	47	60	49	57

\* Between the uniformity coefficient (U) and the spacing coefficient squared (S<sup>2</sup>)

3. Type A curve, or curves approaching it in general type (C, D, E), showed the most regular trends, the highest uniformity coefficients and the lowest range coefficients at reasonable spacings. For square spacings of the sprinkler, the widest possible suitable spacing suggested by Christiansen (4) was 71 per cent of the diameter of the wetted area, which in this case would be 42 × 42 feet. The only curves that showed a uniformity coefficient above 70 at a spacing of 40 × 40 feet were A and E. Water distribution appeared to be somewhat more predictable with Curve A than with any of the other types.

4. With the more uniform types of curve (A to E), square spacing tended to give somewhat higher uniformity coefficients than did rectangular spacings. This can be seen by comparing the 30 × 30 spacings with the means of the 20 × 40 and 20 × 50 spacings, each representing an area of 900 square feet.



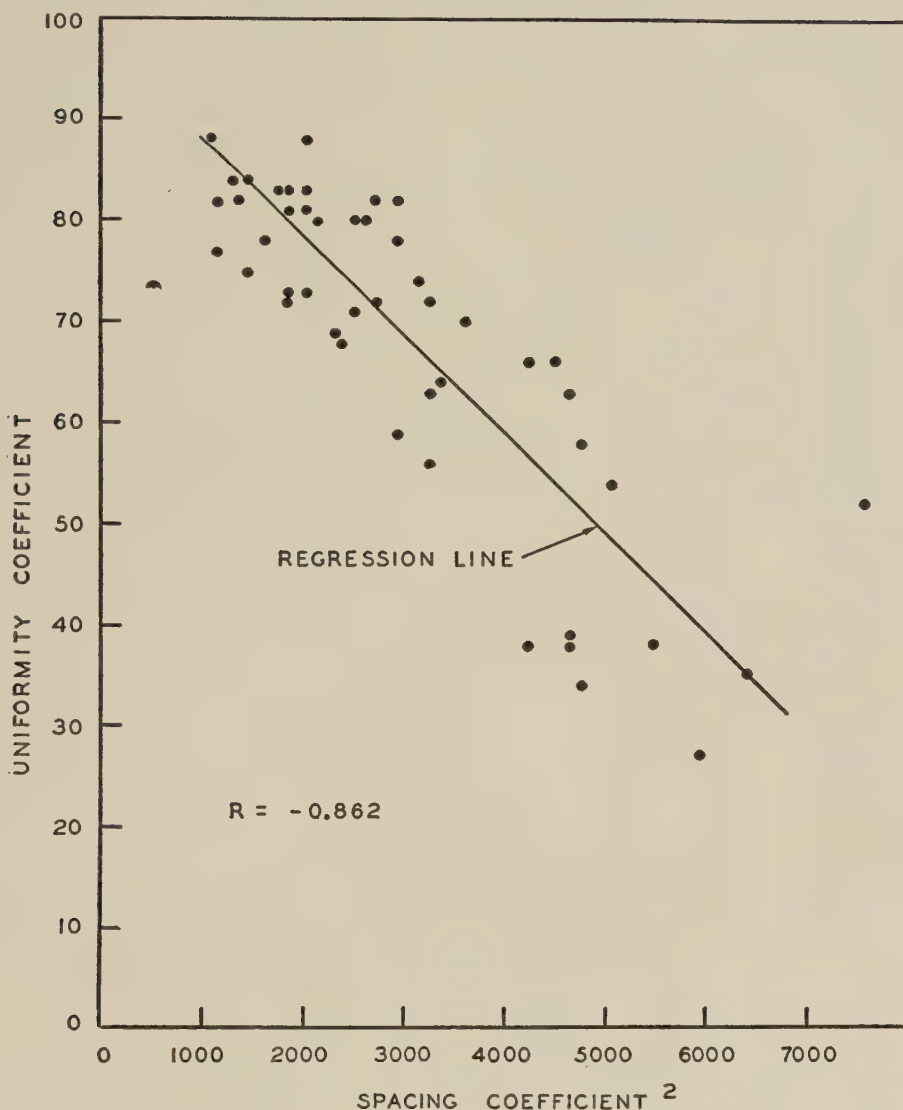


FIGURE 2. Scatter diagram of uniformity coefficient plotted against square of spacing coefficient.

### *Field Test Curves*

The general effect of sprinkler spacing on the uniformity coefficient is illustrated in Figure 2, in which are plotted the data from the water distribution charts obtained with different square spacings from the Type C curves. Each plotted point in this chart represents the  $U$  and  $S^2$  values obtained from a square spacing (such as  $25 \times 25$  feet) charted from a distribution test on a single sprinkler. It will be noted that the uniformity coefficient varied inversely as the square of the sprinkler spacing. It will also be noted that although the trend illustrated was quite a definite one, there was too wide a distribution of the values away from the regression line to

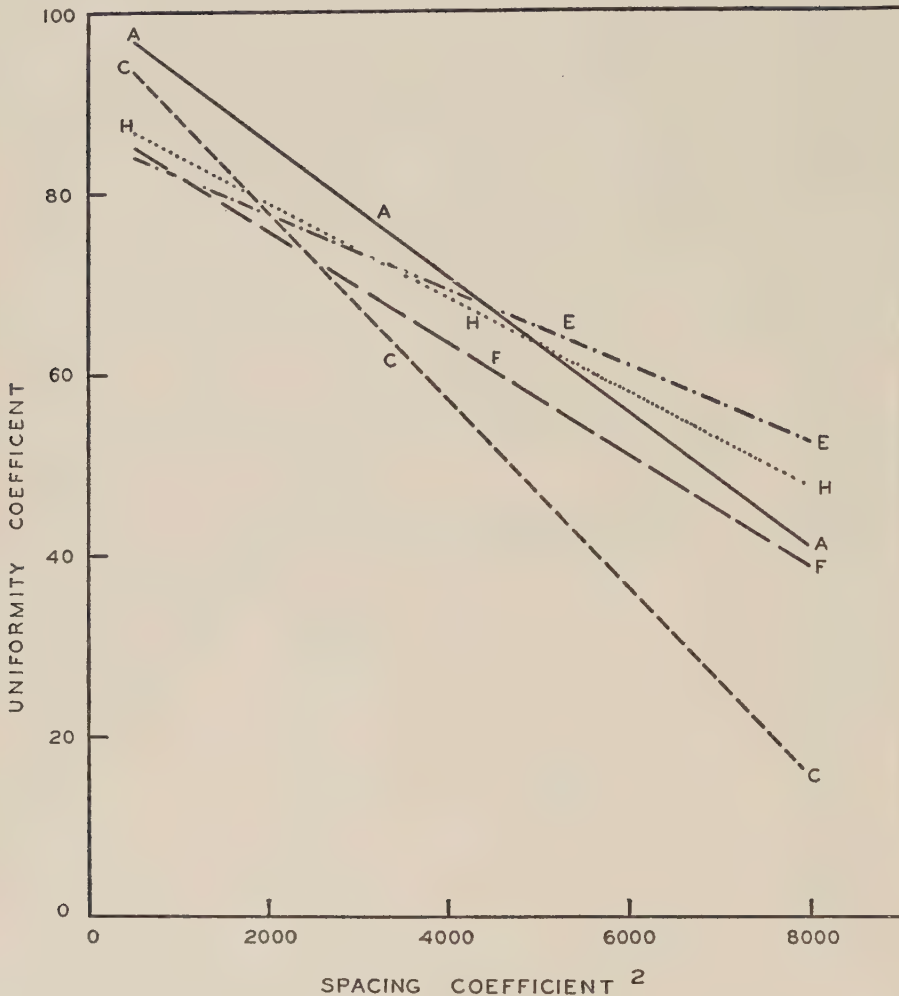


FIGURE 3. Regression lines indicating the relationship between the uniformity coefficient and the sprinkler spacing for each type of curve, as found by field tests.

justify the use of the  $S$  value for determining the probable  $U$  value. This held true for all of the regression lines, both for square spacing and for rectangular spacing. It cannot be considered safe, therefore, to judge the uniformity of distribution entirely by the type of distribution curve.

The regression lines for square spacings for each of the five types of curve studied are shown in Figure 3. Somewhat similar regression lines were obtained with rectangular spacings, though their slopes with increasing  $S$  values were greater in every case than with square spacing. Some of the information obtained from the square-spacing studies is summarized in Table 3. It will be noted that (a) Curves A and C gave somewhat the highest correlations; (b) none of the curves gave an average uniformity coefficient as high as 70 at a spacing of 70 per cent of the diameter of throw; (c) the widest spacing that would give a uniformity coefficient of 70 was



64 per cent of the diameter with square spacing; (d) square spacing gave higher uniformity coefficients in most cases than did rectangular spacing covering the same area, this being especially true with the more regular types of curve, and (e) the wider the sprinkler spacing, the greater was the advantage of square spacing over rectangular spacing. As already noted, all rectangular spacings were based on a 20-foot distance along one side, so that the lower values of S were associated with long and narrow rectangles.

Information obtained in a similar manner from the range coefficients is summarized for the square spacings in Table 4. Those for rectangular spacings were somewhat higher than were those for square spacings. It will be noted that (a) Curve H gave the highest correlation; (b) the best range coefficient at a sprinkler spacing of 70 per cent of the diameter of throw was 135, and (c) the widest spacing that would give a range coefficient as low as 70 was 47 per cent of the diameter. With a range coefficient of 70, the greatest depth of water (after allowing for overlapping) is 2.1 times the least depth; with a range coefficient of 135, the greatest depth is 5.2 times the least.

TABLE 4.—RANGE COEFFICIENT DATA FROM FIELD TESTS

	Type of curve				
	A	C	E	F	H
Number of pairs correlated	60	46	67	58	52
Coefficient of correlation*	+0.77	+0.83	+0.74	+0.82	+0.90
R when S = 70%**	135	149	139	149	152
R when S = 60%	107	121	111	125	122
R when S = 50%	78	91	91	100	88
S in % when R = 70	47	42	41	38	44

\* Between the range coefficient and the sprinkler spacing in percentage of the diameter of throw.

\*\* R = range coefficient.

S = sprinkler spacing expressed in percentage of diameter of throw.

TABLE 5.—COMPARISON OF SOME UNIFORMITY COEFFICIENT AND RANGE COEFFICIENT VALUES

Uniformity coefficients		Range coefficient	Ratio
Wilcox and Swailes	Christiansen		$\frac{\text{highest value}}{\text{lowest value}}$
50	58	173	13.8
60	67	141	5.8
70	76	109	3.4
75	80	93	2.7
80	84	77	2.3
85	88	60	1.9

### *Desirable Uniformity of Distribution*

It has been suggested by Wilcox and Swailes (9) that sufficient uniformity of water distribution should be obtained to produce a uniformity coefficient of at least 70, by their method of calculation. The question arises as to whether this value is normally associated with a reasonable range of values representing water depth; in other words, with a reasonable range coefficient. In answering this question, it was considered desirable to determine the whole relationship between the uniformity coefficient and the range coefficient. To do this, distribution charts were prepared, coefficients of correlation were calculated, and regression equations were calculated. The regression lines were straight lines in all cases. The overall correlation (using 305 pairs) was  $-0.846$ . A curved regression was obtained when the uniformity coefficient (U) was charted against the uniformity coefficient calculated by the method of Christiansen (3). Some of the more pertinent relationships between the uniformity coefficients and the range coefficient are presented in Table 5.

An examination of the distribution charts of these correlations indicated that at any one value of the uniformity coefficient, there was often a wide variation in the range coefficient. If extreme values (especially extremely low values) of depth of water are to be avoided, a reasonably low range coefficient should be aimed at; but this appears difficult to accomplish without likewise aiming at a high uniformity coefficient. In order to obtain a reasonable range of values, it appears necessary to aim at a uniformity coefficient of at least 75 to 80 by the Wilcox and Swailes method, which should give a range coefficient of around 93 to 77.

·18	·34	·46	·45	·27	·18
·32	·41	·45	·45	·34	·31
·48	·47	·48	·44	·44	·48
·46	·42	·45	·45	·41	·46
·30	·40	·45	·43	·32	·29
·20	·36	·46	·42	·27	·18

FIGURE 4. Distribution chart obtained by applying a  $30 \times 30$  foot spacing to a sprinkler test having a distribution curve of the H type (9).



The selection of the exact standards at which to aim should not affect the selection of the most desirable type of distribution; however, it will affect our conception as to the safe distance apart for spacing sprinklers. With the A curve (Figure 1), square spacing of the sprinklers at 64 per cent of the diameter of throw gave, on the average, a uniformity coefficient of 70 and a range coefficient of 109, and spacing them at 58 per cent of the diameter of throw gave a uniformity coefficient of 75 and a range coefficient of 93. Even where the most reliable types of distribution curve are obtained, therefore, the sprinklers should not be placed on the square at a distance greater than 60 per cent of the diameter of throw. An example of a distribution pattern from a Type H curve is illustrated in Figure 4. Each value in this figure represents the depth of water in inches per hour. It will be seen that, in spite of the reasonably close spacing (58 per cent of the diameter of throw), there is rather a wide range of values in this distribution chart: The uniformity coefficient is 75, the range coefficient is 91, and the highest value is 2.7 times the lowest value.

### CONCLUSIONS

The results obtained in this investigation appear to justify the following conclusions:

1. The best type of distribution curve for general application is one showing a steady decrease in rate of water application from the sprinkler out toward the outer circumference of water throw. Irregular curves may provide more uniform water distribution with certain spacings, but cannot be relied on for good general performance.

2. If a sprinkler operating at a certain pressure applies water in such a manner as to give a distribution curve approximating that noted above as being desirable, this does not of itself constitute proof that the water distribution will be satisfactory at any stated spacing. Under field conditions of operation, any one general type of curve can have associated with it rather a wide variation in uniformity coefficients at any one spacing. This is true even when there is no wind at the time of test. It is safer to support the evidence obtained from the type of distribution curve with measurements of uniformity of distribution such as the uniformity coefficient and the range coefficient.

3. To attain reasonable uniformity in water distribution with square spacing of the sprinklers, the sprinkler spacing should be not greater than 60 per cent of the diameter of water throw by the sprinklers. This applies to ideal conditions only; that is, to those cases where the type of curve noted above (in Conclusion 1) is obtained, and where the uniformity of distribution is not affected adversely by wind or by irregular rotation of the sprinkler. Wind, of course, will necessitate placing the sprinklers closer together than this, and preferably in a rectangular pattern with the close spacing at right angles to the direction of the wind (1, 4).

4. On the average, square spacing of the sprinklers gives more uniform water distribution than does rectangular spacing covering the same area. It is true that occasional cases are encountered where more uniform distri-

bution is obtained from rectangular spacing, this being especially so with the less regular types of curve such as Curve F<sub>1</sub>. The longer the rectangle in relation to its width, the less uniform is the water distribution for the same area of coverage.

5. The above conclusions can only be used as a starting-point in making sprinkler recommendations. Various problems are encountered under field conditions that will of necessity affect the recommendation. The greatest of these problems undoubtedly is wind. As already pointed out by other workers (1, 4, 6, 10), the spacing must be reduced in proportion to the amount of wind if any semblance of good distribution is to be retained; and further, under windy conditions the best distribution is obtained by rectangular spacing with the sprinklers spaced more closely together at right angles to the direction of the wind.

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# THE COMPARATIVE EFFECTS OF MANURE AND COMMERCIAL FERTILIZER IN A LONG- TERM SOIL FERTILITY EXPERIMENT

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## ABSTRACT

In a long-term field experiment, 15 tons of manure per acre applied for each cycle of a rotation of mangels, oats, clover, and timothy, was effective in maintaining the yield of each of the crops during a 40-year period. With application of commercial fertilizer containing the equivalent of 64 lb. (N.), 48 lb. ( $P_2O_5$ ) and 37.5 lb. ( $K_2O$ ) per acre for each cycle of the rotation, the yield of mangels was maintained, the yield of oats was increased, but there was some decline in the yield of hay as the experiment was continued. Where a combination of manure and fertilizer was applied at reduced rates, there were trends for the yields of mangels and oats to increase and for the yield of hay to decrease with time. Without manure or fertilizer, the yields of all crops declined markedly with continuation of the experiment.

In a greenhouse test, the yield of alfalfa on surface samples from the plots receiving manure in the field experiment, was significantly higher than that produced on soil from the plots receiving fertilizer only or no treatment.

Chemical analyses of soil samples showed that the experimental area was very variable. In a relatively uniform section of the experimental area, however, values for nitrogen, organic matter, base exchange capacity, and available phosphorus tended to be higher for the manured plots than for those receiving fertilizer only or no treatment.

## INTRODUCTION

A field experiment has been conducted at the Central Experimental Farm, Ottawa, Ontario, from 1911 until the present. The object of the experiment has been to compare the effects of barnyard manure, commercial fertilizer, and barnyard manure plus commercial fertilizer for a rotation of mangels, oats, clover and timothy. Soil samples were taken from these plots in 1951 to measure the relative influence of the field treatments on the yield of alfalfa grown in greenhouse tests with and without phosphorus and potassium fertilizers. In addition, several chemical properties of the soils were determined. The purpose of this paper is to evaluate yield trends, as well as the present fertility status of the plots which have received treatments without modification over a long period.

The soil of the experimental area is a sandy loam, neutral to alkaline in reaction and variable with respect to organic matter. Although the natural drainage of the area varied from poor to moderate, the land has been tile drained. From 1913 to 1952, inclusive, the average annual precipitation was 34.63 inches. The total rainfall for the period April to September inclusive was 18.14 inches, and the average temperature for these months was 58.9° F.

<sup>1</sup> Joint contribution from the Division of Field Husbandry, Soils and Agricultural Engineering, Experimental Farms Service, and the Chemistry Division, Science Service. (Contribution No. 246, Chemistry Division).

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## MATERIALS AND METHODS

The treatments for the rotation were neither randomized nor replicated. Each of the crops—mangels, oats, clover, and timothy—were grown each year. The treatments relevant to this discussion have been designated as  $X_1$ ,  $Y_1$ ,  $Z_1$ ,  $Z_3$  and N, and are shown in Table 1. Considerably more nutrients were supplied by manure than by commercial fertilizer. The manure was well-rotted and was applied on timothy sod in the fall prior to ploughing. The fertilizers were applied broadcast in the spring. Originally the plots receiving the treatments designated as  $X_1$ ,  $Y_1$  and  $Z_1$  were each one acre in size and were located side by side with a set of three plots for each crop in the rotation. In 1921 the  $X_1$ ,  $Y_1$  and  $Z_1$  plots were subdivided to provide for additional treatments, the size of the plots being reduced to one-third acre. At that time the  $Z_3$  plots, which previously received the  $Z_1$  treatment, were introduced as additional check plots. The check plots designated as N were one-third acre in size and were located with the four crops of the rotation side by side at one end of the experimental area.

Soil samples of the 0-6 inch depth were taken in the fall of 1951 from the plots which received the treatments designated in Table 1. No samples were taken from the timothy area since manure had already been applied when the soil samples were collected. These samples, each of which was a composite from 30 locations in a plot, were used for greenhouse and laboratory studies. Composite soil samples of five borings per plot, which were taken from a number of the plots in 1929, were available for examination in the laboratory.

In the greenhouse, the soils were air-dried, passed through a screen with  $\frac{1}{2}$  inch-mesh and placed in gallon pots. An equal volume of soil was placed in each pot. Alfalfa was used as the indicator crop for the experiment. The treatments employed were as follows: (1) check; (2) phosphorus at 80 lb. of  $P_2O_5$  per acre; (3) potassium at 80 lb. of  $K_2O$  per acre, and (4) phosphorus and potassium at 80 lb. of  $P_2O_5$  and 80 lb. of  $K_2O$  per acre. The fertilizer was placed in a layer at a depth of 2 inches. The three groups of pots, representing the three cropping areas (mangels, oats, and clover) were randomized, and the 20 pots comprising the four green-

TABLE 1.—MANURE AND FERTILIZER TREATMENTS PER ACRE IN FIELD EXPERIMENT

Crop in rotation	$X_1$	$Y_1$	$Z_1$		$Z_3^*$	N
	Manure	Fertilizer**	Manure	Fertilizer**	Check	Check
Mangels	tons 15	lb. 100 N + 300 P + 75K	tons 7.5	lb. 50 N + 150 P + 37.5K	—	—
Oats	—	100 N	—	100 N	—	—
Clover	—	100 N	—	100 N	—	—
Timothy	—	100 N	—	100 N	—	—

\*  $Z_3$  same as  $Z_1$  until 1921.

\*\* N—nitrate of soda; P—superphosphate (16 per cent  $P_2O_5$ );  
K—muriate of potash (50 per cent  $K_2O$ ).



TABLE 2.—EFFECT OF MANURE AND FERTILIZER ON THE YIELD OF CROPS IN A FOUR-YEAR ROTATION 1913-1952

Treatment	Average yield per acre											
	Mangels			Oats			Clover hay			Timothy hay		
	1913-1920	1945-1952	1913-1952	1913-1920	1945-1952	1913-1952	1913-1920	1945-1952	1913-1952	1913-1920	1945-1952	1913-1952
X <sub>1</sub> —Manure	tons 20.55	tons 22.20	tons 22.52	bu. 59.7	bu. 61.7	bu. 60.6	tons 3.84	tons 3.83	tons 3.77	tons 2.88	tons 3.12	tons 3.07
Y <sub>1</sub> —Fertilizer	19.00	19.87	20.32	52.6	61.6	59.4	3.60	2.83	3.19	2.56	2.48	2.56
Z <sub>1</sub> —Manure + fertilizer	20.64	25.44	23.04	55.5	62.3	60.0	3.80	3.46	3.52	2.91	2.67	2.78
Z <sub>3</sub> —Check (same as Z <sub>1</sub> until 1921)	—	11.63	13.06*	—	48.4	52.5*	—	2.02	2.68*	—	2.16	2.36*
N—Check	12.59	4.06	6.29	42.3	30.8	39.0	2.08	1.46	1.86	—	1.60	1.75**

\* Average for 1921-1952.  
\*\*Average for 1922-1952; used as pasture until 1922.

house treatments for each of the five field treatments were randomized within each group. The experiment was conducted with two replications. Ten alfalfa plants were grown in each pot. Water was added to the soils as surface applications according to the observed requirements.

In the laboratory the soil samples were passed through a 2-mm. sieve. Analyses for pH, total nitrogen, organic matter, base exchange capacity, and exchangeable potassium, were made by the methods in use in this laboratory (2, 3). In addition, so-called available phosphorus was determined by extraction with  $K_2CO_3$  (1).

## EXPERIMENTAL RESULTS AND DISCUSSION

### *Field Experiment*

The average yields of mangels, oats, clover and timothy for the 8-year periods, 1913–1920 and 1945–1952, and for the 40-year periods, 1913–1952, inclusive, are presented for different fertility treatments in Table 2. On the untreated plots the yields of all crops were considerably lower than those obtained on plots receiving manure, fertilizer or a combination of both. Without manure or fertilizer, there was a marked reduction in the yields for a recent 8-year period as compared with those obtained during the first 8-year period. Weeds contributed considerably to the yield of hay on the untreated plots.

Application of manure or a combination of manure and fertilizer resulted in higher yields of mangels than that obtained with the use of fertilizer only. With the combination of manure and fertilizer, the yield of mangels for a recent 8-year period was higher than that obtained for the first few years of the experiment. Fertilizer only and manure only were each effective, however, in maintaining the yield of mangels with time. The yields of oats during the first few years of the experiment were higher on the plots receiving manure only. As the experiment continued, the yields of oats on the plots receiving fertilizer tended to increase with time and in recent years the yields resulting from the different manure and fertilizer treatments have been similar. The yields of hay on the plots receiving manure only or a combination of manure and fertilizer were higher than those obtained on the plots receiving fertilizer only. With the application of manure only, the yield of hay has been maintained with time, whereas the use of fertilizer only, or a combination of manure and fertilizer at reduced rates, has resulted in some decline in the yield of hay from that obtained during the first few years of the experiment. Assuming that fertilizer alone ( $Y_1$ ) provided considerably lower amounts of nutrients than were supplied by manure ( $X_1$ ), the yields of all crops were relatively good on the plots receiving fertilizer.

The regression lines in Figure 1 show the yield trends for the different treatments with advancing 4-year periods. For example, the average decrease in yield of mangels when this crop was grown every fourth year on a check plot (N), was 1.071 tons per acre, or a decrease of 10.71 tons per acre for the 40-year period. The negative regression coefficients for all crops on the untreated plots (N and  $Z_3$ ), for clover on the fertilized plots ( $Y_1$ ), and for timothy on the plots receiving manure and fertilizer ( $Z_1$ ) were

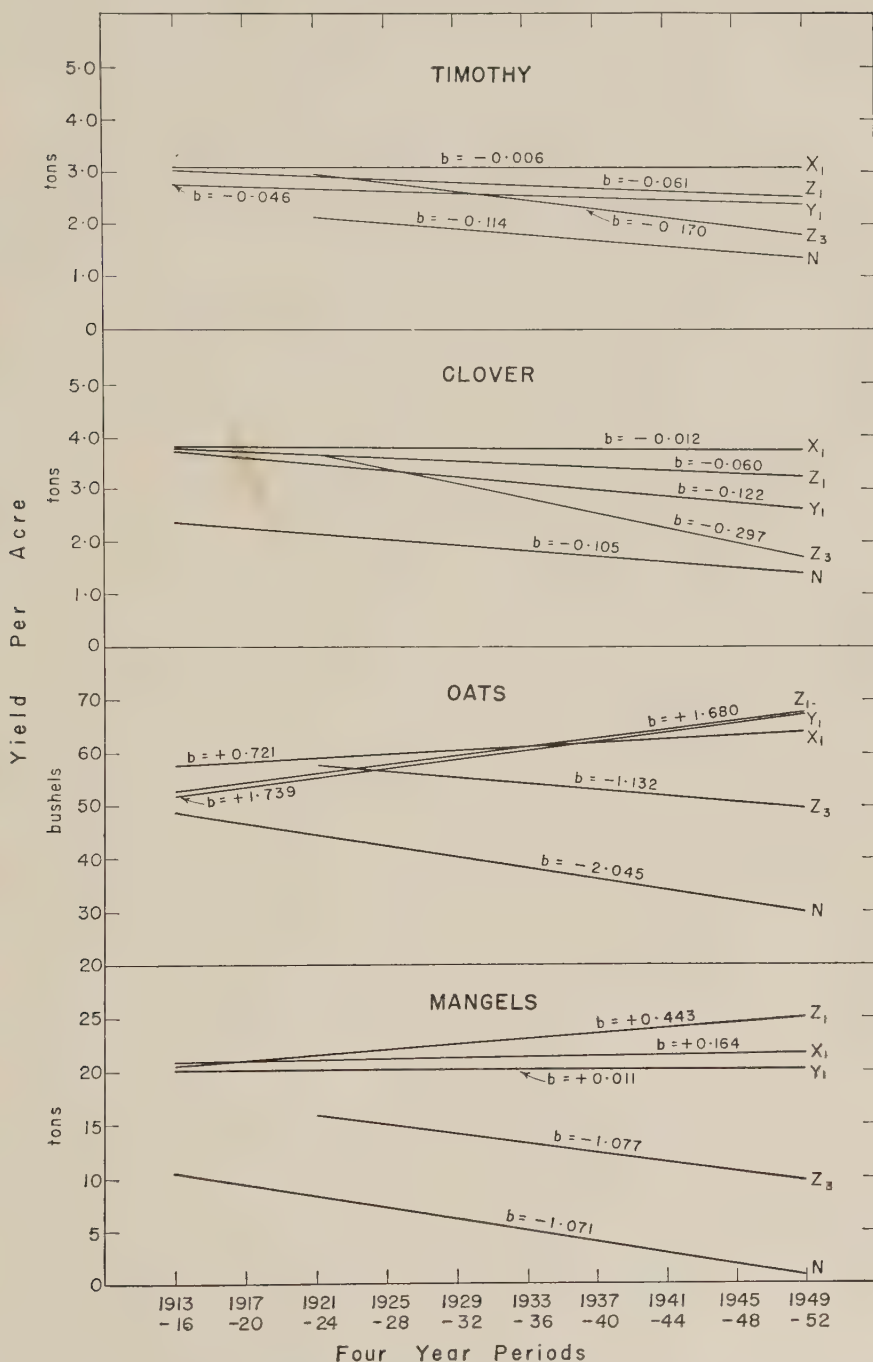


FIGURE 1. Linear regression lines showing yield trends by advancing 4-year periods for various field treatments ( $X_1$ —manure;  $Y_1$ —fertilizer;  $Z_1$ —manure + fertilizer;  $Z_3$ —check;  $N$ —check).



TABLE 3.—GREENHOUSE YIELD OF ALFALFA ON SOILS WHICH RECEIVED DIFFERENT FIELD TREATMENTS  
(Means for 6 one-gallon pots, 2 replications, on soil from mangel, oat, and clover areas; air-dry weight of 4 cuts)

Greenhouse treatments (P at 80 lb. $P_2O_5$ and K at 80 lb. $K_2O$ per acre)	Field treatment			
	$X_1$ —Manure	$Y_1$ —Fertilizer	$Z_1$ —Manure + fertilizer	$Z_3$ —Check*
Check	gm. 52.0	gm. 30.0	gm. 46.7	gm. 26.8
Phosphorus	66.4	43.5	58.0	41.3
Potassium	61.7	36.7	54.7	29.3
Phosphorus + potassium	76.7	55.5	68.6	50.8
L.S.D. (0.05) = 7.95				
				N—Check
				gm. 16.3
				27.7
				21.2
				41.7

\* Same as  $Z_1$  until 1921.

significant. The positive regression coefficients for oats on the fertilized plots ( $Y_1$ ) and on those where a combination of manure and fertilizer ( $Z_1$ ) was applied, were significant.

### *Greenhouse Experiment*

The yields of alfalfa, grown in the greenhouse with and without phosphorus and potassium treatments on soil samples from the field experiment are presented in Table 3. These data show that the different treatments applied in the field over a long period had considerable influence on the yield of alfalfa grown as a test crop. The yields of alfalfa on soil samples from the plots receiving either manure ( $X_1$ ) or manure and fertilizer ( $Z_1$ ) were significantly higher than those obtained on the samples from the fertilized plots ( $Y_1$ ) and the untreated plots ( $Z_3$  and  $N$ ). Soil from the fertilized plots ( $Y_1$ ) tended to produce higher yields of alfalfa than did soil from the untreated plots ( $Z_3$  and  $N$ ), but the differences between the results for the ( $Y_1$ ) and ( $Z_3$ ) samples were not significant. The yields of alfalfa on samples from the manured plots ( $X_1$ ) tended to be higher than those obtained on samples from the plots receiving a reduced quantity of manure and fertilizer ( $Z_1$ ). Regardless of the particular field treatment, application of phosphorus either alone or in combination with potassium in the greenhouse test, resulted in significant increases in the yield of alfalfa over that obtained without phosphorus fertilizer. In the presence of phosphorus fertilizer, application of potassium increased the yields of alfalfa, significantly, over those obtained with phosphorus fertilizer only on each of the samples. Without applied phosphorus, however, the differences in yield resulting from application of potassium were significant only where manure ( $X_1$ ) or manure and fertilizer ( $Z_1$ ) were applied in the field. Application of phosphorus tended to increase the yield of alfalfa to a greater extent than did application of potassium, irrespective of the particular field treatment. An analysis of variance of the data showed that the interaction between the greenhouse and field treatments was not significant.

### *Chemical Analysis*

Some chemical properties of the soil samples used in the greenhouse experiment and of the samples taken in 1929 from a number of the field plots, are presented in Table 4. In particular, the nitrogen, organic matter and base exchange values for the 1929 and 1951 samples present evidence that the experimental area is subject to very great soil variability.

The 1951 sample from the check plot ( $Z_3$ ) in the mangel series showed a marked decline in the values for chemical properties associated with organic matter from those obtained for the 1929 sample. Within the clover series, where the land would appear to have been relatively uniform when the experiment was begun, there was a trend for higher values of nitrogen, organic matter, base exchange capacity and phosphorus to occur for the plots receiving manure or a combination of manure and fertilizer than for those receiving fertilizer only or no treatment.

### *Relation of Exchangeable K and Available P to Yield*

On the basis of the fifteen samples obtained from the plots in 1951, exchangeable potassium and available phosphorus were each correlated with

TABLE 4.—ANALYSES OF SOIL SAMPLES TAKEN FROM FIELD EXPERIMENT  
(Results expressed on an air-dry basis)

Field treatment	pH		Nitrogen (N)		Organic matter		Base exchange capacity		Exchangeable potassium (K)		Phosphorus (P) (K <sub>2</sub> CO <sub>3</sub> method)	
	1929	1951	1929	1951	1929	1951	1929	1951	1929	1951	1929	1951
<i>Clover series*</i> X <sub>1</sub> —Manure Y <sub>1</sub> —Fertilizer Z <sub>1</sub> —Manure + fertilizer Z <sub>3</sub> —Check** N—Check	7.3	7.2	% 0.14	% 0.15	% 3.4	% 3.4	m.e./100 gm.	m.e./100 gm.	m.e./100 gm.	m.e./100 gm.	p.p.m.	p.p.m.
	7.9	7.4	% 0.10	% 0.12	% 2.4	% 2.7	15.4	14.0	0.06	0.08	115	80
		7.8				4.1	13.0	12.0	0.11	0.08	75	44
		7.9		% 0.13		3.1		14.0		0.08		56
		7.2		% 0.12		2.7		12.6		0.11		56
<i>Mangel series</i> X <sub>1</sub> —Manure Y <sub>1</sub> —Fertilizer Z <sub>1</sub> —Manure + fertilizer Z <sub>3</sub> —Check** N—Check	7.4	7.5	% 0.43	% 0.39	% 16.1	% 17.7						
	7.0	7.0	% 0.61	% 0.53	% 24.1	% 23.3	38.8	36.6	0.21	0.21	95	88
		7.0		% 0.62		21.2	51.2	50.3	0.19	0.19	82	120
	7.2	7.5	% 0.61	% 0.36	% 23.7	% 11.6	49.1	52.0	0.25	0.25	78	98
	7.7	7.8	% 0.11	% 0.12	% 2.7	% 2.4	14.7	35.1	0.17	0.17	65	60
<i>Oat series*</i> X <sub>1</sub> —Manure Y <sub>1</sub> —Fertilizer Z <sub>1</sub> —Manure + fertilizer Z <sub>3</sub> —Check** N—Check		7.2		% 0.53		12.4						
		7.7		% 0.40		10.7		39.8		0.25		95
		7.9		% 0.17		4.4		32.3		0.13		62
		8.0		% 0.16		3.4		14.3		0.08		42
		7.7		% 0.12		2.5		15.0		0.11		35
								12.8		0.04		43

\* Group of plots on which the crop designated was grown in 1951.

\*\* Same as Z<sub>1</sub> until 1921.



yield response to applied potassium and phosphorus in the greenhouse. To obtain the yield response to potassium, the yield of alfalfa where phosphorus was applied was expressed as a percentage of the yield obtained where phosphorus and potassium were both used. Similarly, for yield response to phosphorus, the yield where potassium only was applied was expressed as a percentage of that obtained for the pots receiving both phosphorus and potassium. The correlation coefficient for yield response and exchangeable potassium (+ 0.594) was significant at the 5 per cent level and that for yield response and available phosphorus (+ 0.642) was significant at the 1 per cent level.

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# TUBER GRAFT TRANSMISSION OF POTATO LEAF ROLL<sup>1</sup>

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## ABSTRACT

In experiments repeated during three seasons, involving over 1500 tuber grafts of three potato varieties—Carter's Early Favorite, Netted Gem, and Canus—the leaf-roll virus was successfully transferred to healthy sets. Occasionally transmission was successful in 48 per cent of the grafts, and 12 to 20 per cent transmission was fairly common. Core grafts proved to be as effective as grafts secured by binding diseased and healthy sets together. The placing of diseased tissue within one-quarter inch of the eye in healthy sets tended to give the highest transfer. Grafts made from Carter's Early Favorite seemed more successful than those made from Canus or Netted Gem. Transfer of the virus was definitely more successful in grafted sets stored at 40° F. for 26 days than in those stored for 5 days.

## INTRODUCTION

Schultz and Folsom (5), in 1921, bound together freshly cut halves of 20 healthy potato tuber sets with an equal number of sets infected with leaf roll, and obtained transmission of the virus to healthy plants in three of these grafts. Murphy and M'Kay (3), in 1924, made 71 core grafts and from these obtained transfers of leaf roll to five healthy plants, and then only in those sets stored in a cool temperature before planting.

It is possible that healthy cut sets become infected by contact with diseased ones in the bin before they are planted. This method of infection could be of considerable practical importance if the type and duration of contact established between these sets favours the transfer of the virus. Apparently no data have been published to show whether or not such transfer occurs more frequently in some varieties than in others; or whether a relatively short period of contact between the sets is as effective as a longer one; or, finally, whether or not the distance between the growing bud in the healthy set and the point of contact with the infected set is an important factor in transfer of the virus. The aim of the present study was to obtain experimental evidence on these questions.

## METHODS

In one method of grafting, healthy 2-ounce sets having one eye were bound to similar diseased sets having none. An easier method consisted of removing a core about  $7/16$  in. in diameter and approximately  $1\frac{1}{2}$  in. long from a healthy set and replacing it with a slightly larger one taken from a tuber infected with the leaf-roll virus. The first method was originally described by Quanjer (4), and since then has been employed by Schultz and Folsom (5). Goss (2) described the core method. The distance the virus can move through healthy tissue and cause infection was determined in these tests by cutting the healthy sets, in a cool room, in such a manner that the remaining eye in each healthy set was situated  $\frac{1}{4}$ ,  $\frac{1}{2}$ , or 1 inch,

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TABLE 1.—PERCENTAGE TRANSFER OF LEAF-ROLL VIRUS BY TWO TUBER-GRAFT METHODS\*

Year	Variety		Distance (in.) of healthy eye from infected tissue					
	Diseased	Healthy	Infected set bound to healthy set			Infected core inserted into healthy set		
			$\frac{1}{4}$	$\frac{1}{2}$	1	$\frac{1}{4}$	$\frac{1}{2}$	1
1951	Early Favorite**		%	%	%	%	%	%
	Early Favorite	Netted Gem	12	—	—	48	—	—
	Early Favorite	Canus	16	—	—	8	—	—
1952	Early Favorite	Early Favorite	16	—	—	24	—	—
	Netted Gem	Netted Gem	4	—	0	0	0	0
	Netted Gem	Canus	12	—	0	0	0	0
	Netted Gem	Early Favorite	16	—	8	16	0	12
	Early Favorite	Netted Gem	12	—	4	4	4	0
	Early Favorite	Canus	28	—	8	24	16	0
1953	Early Favorite	Early Favorite	20	—	12	8	8	12
	Netted Gem	Netted Gem	0	8	8	—	—	—
	Netted Gem	Canus	0	0	0	—	—	—
	Netted Gem	Early Favorite	0	0	0	—	—	—
	Canus	Netted Gem	4	4	0	—	—	—
	Canus	Canus	4	4	8	—	—	—
	Canus	Early Favorite	4	0	8	—	—	—
	Early Favorite	Netted Gem	32	0	0	—	—	—
	Early Favorite	Canus	16	4	0	—	—	—
	Early Favorite	Early Favorite	40	0	8	—	—	—

\* 25 sets per treatment. Total grafts 1575.  
\*\* Carter's Early Favorite.



respectively, from the tissue infected by leaf roll. The exposed edges of the contacting surfaces were immediately sealed with wax to exclude air, and the sets were planted in field plots within 48 hours after treatment. These plots were planted late in May when, at Edmonton, the mean soil temperature is usually about 54° F. In these experiments conducted in 1951, 1952 and 1953, the varieties Netted Gem, Canus, and Carter's Early Favorite were used in various combinations. Each treatment consisted of 25 grafts; a total of 1575 grafts was made for all treatments during the three years. The tubers used in these studies were stored from October to April at about 35° F. and were in excellent condition for the experiment. To ensure that each tuber carried the leaf-roll virus, the tubers of the diseased stock were indexed before they were used for grafting. The healthy tubers were indexed at the time of grafting. The varieties used, of both healthy and diseased tubers, are indicated with the data recorded in Table 1. Evidence of successful virus transmission was the appearance of severe symptoms of leaf roll in the young growing plant. The records were taken prior to mid-July, before symptoms of field infection caused by viruliferous aphids developed. The foliage of the plants in these experiments was effectively protected from aphids throughout the season by weekly applications of a suitable insecticide.

### RESULTS AND DISCUSSION

Although there were some variations, the method of binding together diseased and healthy sets apparently gave the most consistent results.

The data in Table 1 indicate that the leaf-roll virus ordinarily does not move quickly in a healthy tuber because the infection spread more often when the eyes were placed  $\frac{1}{4}$  inch from the diseased tissue than when they were placed at a distance of 1 inch. Apparently the highest percentage of transfers is obtained when the healthy eye is very close to the virus source.

The leaf-roll virus was transferred more frequently when the variety Carter's Early Favorite was used as the diseased tissue in the graft than when either Netted Gem or Canus was employed. No explanation can be offered for this difference in behaviour of the varieties. Transfer of the virus seems to depend upon successful union of the phloem elements and it is possible that their union occurs more readily in certain varieties than others. Successful union in potato stems appears to require approximately 10 to 12 days at a temperature of about 65° F. (1).

Murphy and M'Kay (3), who reported only five successful transfers out of 71 core grafts, obtained transmission only in those stored in a cool temperature before planting, and concluded that the low temperature might be more favourable than the higher one which was included in their experiment. On the assumption that transfer is aided by slow suberization of the core graft, an experiment was carried out to test the effect of temperature and length of storage on the sets. Seventeen sets of the Carter's Early Favorite variety were core-grafted, stored for 26 days at 40° F., then planted in the greenhouse in soil at a temperature of about 65° F. Another lot was grafted and stored at 40° F. only 5 days before being planted. It is significant that the sets held for 26 days produced leaf roll in 47.1 per cent of the plants, whereas those stored for 5 days produced leaf roll in 22.7 per cent of the plants. The controls were healthy (Table 2).

TABLE 2.—EFFECT OF LENGTH OF STORAGE ON TRANSMISSION OF LEAF-ROLL VIRUS BY CORE-GRAFTED POTATO TUBERS AT GREENHOUSE TEMPERATURE OF 65° F., FOLLOWING STORAGE AT 40° F.

Test	Days' storage 40° F.	Number of plants	Leaf-roll plants
A	26	17	<sup>%</sup> 47.1
Control	26	25	0
B	5	44	22.7
Control	5	25	0

The data show that if the conditions are favourable the leaf-roll virus can be transferred fairly readily by grafting diseased tissue into healthy sets. Therefore, it is possible for healthy cut sets stored on the farm one week or longer to become infected with the leaf-roll virus if the sets come in proper contact with diseased cut sets at favourably low temperatures. However, since the sets are usually planted within a day or so after being cut, the danger of their becoming infected with the leaf-roll virus is practically eliminated.

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# THE NUTRITIONAL VALUE OF RAPESEED OILMEAL: A REVIEW<sup>1</sup>

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## ABSTRACT

A comprehensive review of rapeseed oilmeal is given under the headings (1) evidence for toxic factor (2) mode of action of toxic factor (3) counter-action of toxic factor (4) nutrient content and (5) current status of rapeseed oilmeal in practical livestock and poultry feeding. The bibliography contains 65 references.

## INTRODUCTION

Canadian interest in rapeseed oilmeal is of comparatively recent origin, since the production of rapeseed in Canada did not assume much commercial importance until the Second World War. The first report on the feeding value of rapeseed oilmeal by workers on this continent appeared in 1944, although the first experiments on the subject appear to have been published in Germany as long ago as 1872.<sup>3</sup> Numerous other reports have followed these to add evidence that rapeseed oilmeal contains one or more toxic factors. Findings differ, however, as far as the nature, the significance and the control of the toxic<sup>3</sup> factor are concerned. It is, therefore, the purpose of this report to present and summarize the present status of this problem.

## THE EVIDENCE FOR PRESENCE OF A TOXIC FACTOR

In 1901 Sjollesma (56) identified crotonylisothiocyanate as a constituent of the essential oil fraction of rapeseed. Viehover *et al.* (61), in 1920, working on methods for identifying rapeseed in importations, found crotonyl- and allylisothiocyanates to be present in rape and mustard seed, respectively, and established their relative toxicities in rabbits, although the technique employed was of questionable value with respect to the present rapeseed oilmeal problem. In 1941 Kennedy and Purves (31) in New Zealand reported hyperplastic changes in the thyroid, and changes in the pituitary similar to those following thyroidectomy, as a result of feeding *Brassica* seeds to rats. Delayed maturation of ovaries in immature rats was also reported.

Pettit *et al.* in 1944 (46) reported that, while 14 per cent rapeseed oilmeal in chick starters was satisfactory, considerable mortality resulted when the level was raised to 20 per cent.

In 1948 Blakely and Anderson (12) reported enlargement of thyroid glands to the extent of five or six times normal size in turkey poult fed rations containing up to 20 per cent rapeseed oilmeal. In the same year Turner (58) reported marked goitre and growth depression in chicks fed diets containing 40 per cent rapeseed oilmeal. Allen and Dow (2) reported thyroid hyperplasia in chicks coincident with some improvement in gains and feed efficiency.

<sup>1</sup> Prepared at the request of National Research Council Committees on Grain Research and Animal Nutrition. Contributed at the invitation of the Editorial Board.

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<sup>3</sup> The term 'toxic' as used in this report means productive of varying degrees of abnormality with respect to growth, thyroid size and/or its histology and several other disturbances of tissues or functions.



Clandinin<sup>1</sup> has recently reported deleterious effects from the use of rapeseed oilmeal in chick rations, with Argentine type rape being more toxic than Polish type. In 1953 Bell and Williams (10) found that mice grew poorly when rapeseed oilmeal was the main source of protein in the ration, and the same workers later<sup>2</sup> reported possible disturbances in females during lactation: Van der Veen and Hart (60) reported depressed appetites resulting from inclusion of rapeseed oilmeal in poultry rations. Witz (63) also obtained deleterious effects from feeding higher levels of the oilmeal to poultry.

In 1949 Astwood *et al.* (5) and Carroll (18) isolated a goitrogenic substance from *Brassica* seeds, including rapeseed, that was later proven to be 1-5-vinyl-2-thiooxazolidone, and which has since been synthesized (22). In the same year as thiooxazolidone was found to be present, Matet *et al.* (42) isolated the glucosides sinigrin and gluconapin from rapeseed; these substances are precursors to allyl- and crotonyl-isothiocyanates. Shortly afterwards the possible relationship between the isothiocyanates and thiooxazolidone was pointed out (5). Dow and Allen (21) concluded that the toxic factor was a thiocyanate because of the type of response obtained to iodide in the diets of chicks. Wetter and McConnell<sup>3</sup> found isothiocyanate contents (expressed as allylisothiocyanate) varying from 0.3 to 1.4 per cent in solvent-extracted rapeseed oilmeals. Samples of Polish, Argentine and Turkish types were tested, and the highest values were found in the Turkish samples. Raciszewski (49) isolated thiooxazolidone from rapeseed and later synthesized the substance.

In studies with swine Nordfeldt *et al.* (43) found that the use of 10 to 20 per cent rapeseed oilmeal in the ration resulted in decreased growth rates and enlargement of the liver, kidney and thyroid. Seale<sup>4</sup> reported satisfactory feedlot performance in swine but found evidence of thyroid disturbances due to the inclusion of rapeseed oilmeal.

A single Swedish reference (26) indicates that rapeseed oilmeal is suitable for dairy cows, but emphasizes that it must be fed dry.

There is thus evidence based on experiments with rats, rabbits, chicks, mice, turkey poults, swine, and possibly cattle, as well as chemical data, to establish the fact that some toxic factor (or factors) exist in rapeseed oilmeal.

#### THE MODE OF ACTION OF THE TOXIC FACTOR

Goitrogenic substances are not restricted to rapeseed, nor, for that matter, to the *Brassica* family, nor does the evidence indicate that the same factor is involved in all members of the *Brassica* family. In addition, there is some suspicion on the part of a few investigators (10, 20, 42) that more than one toxic factor may be involved, since extensive kidney, liver, adrenal and growth involvements have been observed.

Some information on mode of action has developed from iodine studies. Marine *et al.* (39) found dietary iodine to be effective against the cabbage goitrogen for rabbits. Kennedy *et al.* (31) and Purves (48), however, found this element to be only partially effective against the rapeseed factor fed to

<sup>1</sup> Clandinin, D. R. *Private communication.* 1955.

<sup>2</sup> Bell, J. M. *Unpublished.*

<sup>3</sup> Wetter, L. R., and W. McConnell. *Private communication.* 1955.

<sup>4</sup> Seale, M. *Private communication.* 1955.

rats. Iodide appeared to reduce gland enlargement, but the hyperplastic condition persisted. In 1933 Spence (57) reported a species difference in that chickens were less sensitive than rabbits to methyl cyanide as a goitrogen. Astwood (6), in reviewing the problem in 1949, concluded that the mechanism of goitrogenesis from rapeseed is the same as that elucidated for the sulphonamides and thioureas; that is, the primary effect is one of thyroid inhibition. The interference with thyroxine synthesis leads to increased synthesis of thyrotropin which results in hyperplasia. He was of the opinion that 1-5-vinyl-2-thioxazolidone was the rapeseed goitrogen.

Rosenburg (51), in discussing further the action of goitrogens, stated that "... most classes of antithyroid substances are either competitive substances or inhibitors of peroxidase, the thiocarbamates being in the former category and the sulphonamides, anilines and polyphenols in the latter."

Three reports direct attention to possible effects of 'goitrogens' that are exerted directly on tissues and independently of thyroid function. Bach (8) found a definite retardation of tissue oxidation of ascorbic acid when either allylthiocyanate or sinigrin was present in concentrations of  $5.7 \times 10^{-4}$ . Benda (11) reported that respiration in guinea-pig liver slices was depressed by 50 per cent by small concentrations ( $10^{-2}$ M) of allylthiocyanate. Inhibition of dehydrogenation reactions involving pyruvic, lactic, citric and succinic acids by allylthiocyanate was shown by Flichenstein and Berg (25).

Reports on interactions between sexes and goitrogens are contradictory. Absence of sex differences in response to goitrogens, or failure of androgens and estrogens to influence thiourea or thiouracil responses in rats, were indicated by the reports of MacKenzie (38); Segaloff (54); and Goldsmith (29). On the contrary, Marine and Baumann (41) found male rats to be better able to withstand the toxicity of thiourea than females and with mice Bell<sup>1</sup> found females better able than males to tolerate the toxic effects of rapeseed oilmeal.

Recalling evidence for species and sex differences, Dow and Allen's (21) conclusion that a thiocyanate is involved, Astwood's (6) opinion that thioxazolidone was the active agent, and considering that Bell<sup>1</sup> obtained no growth depression in mice fed diets containing 0.002 per cent added thioxazolidone, it can only be concluded that more research is needed. The postulation by Pitt-Rivers (47) that isothiocyanates and thioxazolidone are closely interrelated is worthy of serious consideration. The sex and species effects possibly deserve controlled study with respect to sex differences in thyroxine output, and with respect to the influence of (metabolic) body size on basal metabolic rate and thyroxine output.

#### COUNTERACTION OF THE TOXIC FACTOR

Goitrogenic materials in cabbage appear to be rendered harmless by sodium iodide fertilizer applied to the growing plant (14) or by the addition of iodide to the diet (39, 55). Similarly, the effects of goitrogenic materials contained in linseed oilmeal and soybean oilmeal are nullified by iodide (18, 34, 64). On the other hand, only partial correction, if any, of the

<sup>1</sup> Bell, J. M. *Unpublished*.

rapeseed goitrogen by iodide has been obtained by most workers (2, 31, 35, 48). Dow and Allen (21), however, on the basis of work with chicks, concluded that rapeseed oilmeal can replace soybean oilmeal in the ration of broilers provided sufficient iodide is present.

Whereas most reports indicate unsatisfactory responses to iodide, some success has resulted from the use of thyroxine or thyroproteins. Purves (48) obtained inhibition of thyroid hyperplasia in rats by using thyroxine doses as small as 3 micrograms/100 gm. body weight daily. Blakely and Anderson (13) fed iodinated casein 'protamone' as 0.022 per cent of turkey poult diets and obtained 'more normal' growth rates and thyroid sizes by this means. The diets contained 20 per cent rapeseed oilmeal. Kratzer *et al.* (35) used 0.01 per cent protamone and effectively reduced thyroid weights but obtained little effect on gains. The results of including protamone in rapeseed oilmeal rations for mice have been less encouraging<sup>1</sup>. A level of 0.5 per cent protamone resulted in severe growth depression and levels of 0.1 and 0.01 per cent were ineffective. Nordfeldt *et al.* (43) obtained only partial counteraction of the toxic factor(s) by iodinated casein in swine experiments. On the other hand, the latter workers found repeated water extraction *in vacuo* at 100° C. to be an effective method of removing most of the toxic principle.

Antigoitrogenic substances were found in certain plants by Marine *et al.* (39), who stated that such materials probably have thyroid sparing action by providing another mechanism for promoting tissue oxidations.

A degree of improvement in nutritional quality has resulted from water extraction procedures (2, 10, 47). Steaming (30) and cooking (62) have also been claimed as effective measures for destroying the toxic factors. In experiments with mice, autoclaving of rapeseed oilmeal was ineffective by itself but a more favourable response to antibiotic-B<sub>12</sub> supplements was obtained after steam heating<sup>1</sup>. Of interest is the finding in this laboratory that the re-incorporation of aqueous extracts of the meal, following soaking, steam heating or mild HCl hydrolysis, resulted in more serious growth depression than that which resulted from use of the untreated rapeseed oilmeal.

In summarizing the attempts to counteract or remove the toxic factor, the following are methods that have been attended by some success:—cooking, steaming, addition of iodide, addition of protamone, steaming plus use of antibiotics, water extraction and limiting the amount of rapeseed oilmeal in the ration. The majority of the researches suggests that limitation of use is the only satisfactory and practicable method yet available to counteract the total effect of the rapeseed oilmeal factor.

#### THE NUTRIENT CONTENT OF RAPESEED OILMEAL

Aside from the question of a toxic principle, the nutrient content of rapeseed oilmeal is of interest. The earliest work on this matter appears to have come from Germany in 1872 and 1898 in a report from Kuehn *et al.* (36) on composition and digestibility of rapeseed oilmeal by steers and one from von Knieriem (32) on sheep. These reports were followed by the works of Woehlbier (65) on sheep, Lander and Dharmani (37) on cattle and

<sup>1</sup> Bell, J. M. *Unpublished.*



Burkitt (15) on sheep. A summary of the relevant data, as recorded by Schneider (52), is given in Table 1, from which it is apparent that rapeseed oilmeal differs from linseed and soybean oilmeals primarily in Total Digestible Nutrients (T.D.N.). Detailed examination of the digestibility data with sheep and cattle reveals consistently lower digestion coefficients for organic matter in rapeseed oilmeal as compared to linseed and soybean oilmeals. Most of this effect is attributable to the nitrogen-free extract fraction and some to the crude fibre. The average protein digestibility coefficients were comparable to the other oilmeals, being 82 to 86 per cent.

Burkitt (16) reported calcium contents of 0.51 and 0.61 per cent, and phosphorus contents of 0.80 and 0.84 per cent in rapeseed oilmeal; thus it contains more calcium and about the same amount of phosphorus as typical expeller process linseed oilmeal.

Some information is available on protein 'quality' or amino acid content. The amino acid composition has been compared with the amino acid requirements of chicks in Table 2, both expressed as percentages of the protein content and using 20 per cent protein as the protein level in chick rations. It, therefore, appears that rapeseed oilmeal, when used as the only source of protein, would supply adequate levels of all essential amino acids with the possible exception of phenylalanine. Kratzer *et al.* (35) concluded that rapeseed oilmeal was an adequate source of amino acids for chicks, but some of their earlier success with fishmeal used in conjunction with rapeseed oilmeal led to the eventual finding that lysine might be a limiting factor when rapeseed oilmeal was used in practical rations. They found a combination of lysine and protomone to be of real value as a supplement of rapeseed oilmeal in both chick and poult rations. On the other hand, Dow and Allen (21) were able to ascribe the value of fishmeal to its iodine content.

No data have been found on vitamin content of rapeseed oilmeal, but the information cited above suggests that rapeseed oilmeal does not differ materially in nutrient content as compared to vegetable oilmeals in general. It would be of interest to determine whether the toxic factor(s) is involved in the relatively low digestibility coefficients of certain fractions of the meal.

TABLE 1.—COMPOSITION OF RAPESEED OILMEAL COMPARED TO  
LINSEED AND SOYBEAN OILMEALS

	Water	Ash	Crude protein	Crude fibre	N.-F.E.	Crude fat	D.C.P.	T.D.N.
Rapeseed oilmeal								
—Sheep	11.3	7.4	34.7	9.7	30.3	6.6	28.5	63.3
—Cattle	11.6	6.9	30.9	9.0	30.0	11.6	26.5	73.5
Linseed oilmeal	9.0	5.6	35.4	8.2	36.0	5.8	30.8	77.2
Soybean oilmeal	9.1	6.0	44.3	5.7	29.6	5.3	37.2	78.4

TABLE 2.—AMINO ACID CONTENT OF RAPESEED OILMEAL (EXPRESSED AS PER CENT OF PROTEIN,  $N \times 6.25$ )

	Roche and Michel <sup>1</sup> , 1946	Agren <sup>1</sup> ., 1952	Wetter and McConnell <sup>2</sup> , 1954	Chick reqts., 1954
Arginine	6.6	5.6	7.2	6.0
Histidine	—	2.6	—	0.75
Isoleucine	—	3.7	4.5	3.0
Leucine	6.9	5.7	8.7	7.0
Lysine	—	3.5	5.4	4.5
Methionine	—	1.1	5.3	4.0
Phenylalanine	1.9	4.0	—	8.0
Tryptophane	1.2	2.0	—	1.0
Valine	4.2	5.7	6.5	4.0
Threonine	3.3	3.8	4.8	3.0
Cystine	2.4	1.7	1.9	(1.75)
Tyrosine	—	2.3	6.6	(3.5)
Alanine	3.2	1.9	5.0	—

<sup>1</sup> See "References". <sup>2</sup> *Private communication.* 1955.

#### CURRENT STATUS OF RAPESEED OILMEAL IN PRACTICAL FEEDING

In view of the fact that rapeseed oilmeal is currently being used for various classes of livestock and poultry, a summary of findings pertaining to each species is desirable.

##### *Poultry*

Goitrogenic effects, antithyroid effects and growth inhibition have been consistent findings when rapeseed oilmeal has been used at high levels, up to 40 per cent of the diet. Inhibition of growth has been found with levels as low as 10 per cent (23, 63) but levels below 10 per cent have been fed with no apparent ill-effects<sup>1</sup> (26, 33).

Only partial protection has been obtained from the use of protamone (13, 35, 48) and conflicting evidence exists regarding the value of iodide<sup>2</sup> (21, 48), with some investigators claiming no value from potassium iodide, some claiming partial effects and some claiming sufficient effect to allow rapeseed oilmeal to be fed in broiler rations to the extent of 17 per cent. A preliminary report from O'Neil<sup>3</sup> indicates reasonably satisfactory responses in laying hens fed up to 10 per cent rapeseed oilmeal in rations containing soybean oilmeal, meat meal and fish meal. No one has reported palatability problems with poultry.

<sup>1</sup> Clandinin, D. R. *Private communication.* 1955.

<sup>2</sup> Blakely, R. M. *Private communication.* 1955.

<sup>3</sup> O'Neil, J. B. *Private communication.* 1955.

In summary, the use of more than 10 per cent rapeseed oilmeal in poultry feeds would seem inadvisable until more research has been done, and there is some evidence that even this level may be too high for use in turkey rations.

### *Sheep and Cattle*

Rapeseed oilmeal has been fed to weaned lambs in limited amounts<sup>1</sup> (45) and to pregnant ewes (9) with no apparent ill effects, although palatability was a problem. Its digestibility, as mentioned previously, is somewhat below that of linseed and soybean oilmeals but is satisfactory.

With cattle, Burkitt *et al.* (16) found that rapeseed oilmeal could be fed to beef cattle at levels up to 2 lb./head daily with no obvious ill effects. This applied to calves, yearlings and cows-in-calf. Approximately 5 per cent rapeseed oilmeal has been used in dairy mixtures<sup>2</sup> without difficulty and Folke (26) found that at least 2-3 kgm. (4-6 lb.) of rapeseed oilmeal could be fed daily per cow, provided it was fed dry. Palatability was apparently the chief problem in the wet meal but the toxic nature of the aqueous extracts noted previously might well be recalled. Allen and Dow (2) reported that steers would tolerate up to 25 per cent of their ration as rapeseed oilmeal. A bitter substance occurring in rapeseed has been identified as sinapine (53).

These findings suggest that ruminants are less susceptible than other classes of livestock to the effects of the toxic factor(s) in rapeseed oilmeal.

### *Swine*

Seale reported<sup>3</sup> that up to 20 per cent rapeseed oilmeal was used in swine rations without adverse effects on growth rate, feed efficiency or carcass quality, but that there was histological evidence of thyroid disturbance. Nordfeldt *et al.* (43), however, obtained growth depression at 10 and 20 per cent levels as well as other evidence of toxic effects in the internal organs. Bell<sup>2</sup> undertook to evaluate a ration containing 17 per cent rapeseed oilmeal with meat meal, antibiotics and potassium iodide as compared to a standard ration. The pigs receiving rapeseed oilmeal gained more rapidly than the controls until the experiment was terminated on account of the development of rhinitis symptoms in the rapeseed lot. Anderson and Hurwitz (3) found that allyl isothiocyanate was effective against *Ascaris lumbricoides* (round worm) in swine (*in vitro*).

Grussendorf<sup>4</sup> referred to the successful use of rapeseed oilmeal in silage for pigs.

No reports appear to have been published regarding the effects of rapeseed oilmeal in gestation rations for sows.

In summary, therefore, it would seem permissible to use rapeseed oilmeal to the extent of up to one-third of the protein supplement in the rations of growing and finishing market hogs. Insufficient data preclude the formulation of recommendations for other classes of swine.

<sup>1</sup> Blakeley, R. M. *Private communication*. 1955.

<sup>2</sup> Bell, J. M. *Unpublished*.

<sup>3</sup> Seale, M. *Private communication*. 1955.

<sup>4</sup> Grussendorf, W. *Private communication*. 1953.



## SUMMARY AND CONCLUSIONS

In summarizing the current status of the rapeseed oilmeal problem it can be stated that one or more toxic factors exist in this product. Both *l*-5-vinyl-2-thiooxazolidone and isothiocyanates have been isolated and precursors of the latter have also been found. The formation of thiooxazolidone from isothiocyanate has been postulated as a simple oxidation reaction probably facilitated by enzymatic action. These two goitrogens do not behave alike *in vivo*. Iodine has proven to be reasonably effective against thiocarbamide substances. There is a distinct possibility that the toxic factor(s) in rapeseed oilmeal exerts some of its effect at the tissue level where interference with oxidation and/or dehydrogenation reactions may occur. This may increase the demand on the thyroid gland, which in turn may be inhibited by the same or another substance.

Attention is also drawn to the fact that the toxic factor(s) is bearing upon the endocrine system which controls metabolic rate and which in turn is modified by such factors as sex, age, species, breed, strain, environment and diet. To this should be added the peculiarities of the digestive systems in the different species, since these could influence the amounts and types of toxic substance assimilated. There is an urgent need for basic research in these areas.

From the standpoint of practical feeding, the consensus would seem to allow the use of rapeseed oilmeal in the rations of livestock and poultry but with definite restrictions placed on the amounts used according to species of animal involved. In no species does it seem advisable to incorporate more than 10 per cent of rapeseed oilmeal in the total ration in the light of present knowledge, and in certain species, as previously indicated in the report, even this level may be excessive.

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# RELIABILITY OF PREDICTION TESTS FOR MALTING QUALITY OF BARLEY<sup>1</sup>

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## ABSTRACT

The efficiency of prediction tests, which are described, for selecting high quality malting lines has been rigorously tested. The results of prediction tests made on 243 hybrid lines during the years 1949 to 1953 were compared with the results of malting tests made on the same lines grown in a following year. Of those lines later shown to have favourable malting quality, the prediction test selected 79 per cent. The remaining lines with good quality had shown low barley saccharifying activity and their utility had been doubted. The parentage of the majority of these latter lines included Peatland which is known to cause underestimation of malt saccharifying activity. This characteristic emphasizes the need for consultation between plant breeder and chemist.

## INTRODUCTION

A method for predicting the malting quality of barley hybrids early in the plant breeding program and without the necessity of actually malting the material has many advantages. An early estimate of hybrid quality is valuable to the plant breeder in that the time, labour, space and money, consumed in increasing amounts to the stage where it can be malted, are saved if the barley is found to be unsuitable at an early stage of the breeding project. The plant breeder, in possession of advice as to which hybrids he may discard, is able to concentrate on a reduced population that has shown evidence of promising quality or direct the effort saved towards another crossing program.

The prediction test discussed in this paper employs much simpler apparatus, needs far less time, and requires less grain than is necessary for the laboratory malting test. Therefore, the laboratory is able to make more prediction tests than malting tests per man-hour and so to increase the amount of information and assistance given to the plant breeder. The methods at present in use for predicting the malting quality of barley in Canada were developed some time ago. Consequently a review of their development, a description of the techniques employed, and a survey of their value are useful at this time.

The relations between various barley and malt properties have been investigated and discussed (2, 4, 7, 10 and 11) with a view towards the simplification of preliminary tests for malting quality. Sallans and Anderson (9) noted the relation between barley saccharifying activity, as determined by activation with papain, and malt saccharifying activity. Subsequently, tests were made by Meredith, Sallans and Rowland (8) on the utility of the barley saccharifying determination as a method of selecting hybrids with high malt saccharifying activity. Similarly, Meredith (5) tested the value

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TABLE 1.—DESCRIPTION OF HYBRID LINES AND EVALUATION OF MALTING QUALITY

Set No.	Parental varieties	Evaluation <sup>1</sup>				
		—	+	++	+	Total
1	Montcalm, Newal, Peatland, Plush	5	5	2	0	12
2	Titan, Mensury, Newal, Peatland	4	1	2	0	7
3	Montcalm, O.A.C.21 <sup>2</sup> , Newal, Peatland	0	1	14	5	20
4	O.A.C.21 <sup>2</sup> , Montcalm <sup>2</sup> , Peatland <sup>2</sup> , Trebi, Mensury	0	2	14	4	20
5	O.A.C.21, Peatland <sup>2</sup> , Olli, Montcalm, Newal	1	0	1	0	2
6	O.A.C.21, Peatland, Montcalm, Titan	0	0	1	1	2
7	Montcalm, O.A.C.21 <sup>2</sup> , Trebi, Newal, Peatland	1	1	6	2	10
8	O.A.C.21, Newal, Peatland, Olli, Montcalm	8	8	3	2	21
9	O.A.C.21, Newal, Peatland, Montcalm	0	0	8	0	8
10	World Barley Collection varieties	12	0	0	0	12
11	Montcalm, O.A.C.21, Mensury	1	2	9	2	14
12	Montcalm, Peatland	0	1	12	2	15
13	Montcalm, MC 8129	0	0	5	3	8
14	Common 6-Row, MC 8129 <sup>3</sup> , Peatland	2	0	0	5	7
15	O.A.C.21, Vantage, Montcalm, Titan	3	1	5	2	11
16	Newal, Regal, Olli <sup>2</sup> , Peatland, Chevron	6	0	4	6	16
17	Montcalm, Feebar	1	0	0	2	3
18	O.A.C.21, Bolivia, Chevron	0	0	2	3	5
19	Montcalm, Fort, Velvon 11	1	0	0	4	5
20	Miscellaneous	0	0	0	5	5
21	Stephan, Galore, O.A.C.21 <sup>3</sup>	8	0	1	1	10
22	Stephan, Galore, Montcalm	4	0	0	10	14
23	Montcalm, Galore, Duplex	11	2	2	1	16
	Total	68	24	91	60	243

<sup>1</sup> See Figure 1.

of a barley extract determination as a means of selecting lines with high malt extract yield. It was shown that there are high correlations between barley and malt saccharifying activities and between barley and malt extracts.

Prediction tests have now been in use for several years. The accumulation of a fairly large body of data on subsequent malting tests presented the opportunity to test the validity of the prediction test by comparing the

analyses of barley and malt. The test is rigorous, since the results of one year's prediction tests are compared with malting tests made on the same hybrids grown in a following year. The inclusion of material that had appeared unsuitable when tested by the prediction method, but which was not discarded by the plant breeders, facilitated one aspect of the examination. It is possible that the plant breeders wished to use these barleys as parental material in further hybridization.

The prediction test was not developed with the intention of replacing the malting test, but to assist the plant breeder in making an early assessment of his hybrid lines. The malting test is necessary as one of the means of deciding on a final evaluation of malt quality.

### MATERIALS

The study was made using data on 243 barley hybrid lines which represented a wide variety of parental material and which originated at six plant breeding institutions in Canada. The hybrids were submitted for prediction test and were selected by the plant breeder to be grown and malted in a following year. The 243 samples are those on which both sets of data are available between the years 1949 and 1953. The prediction test samples consist of 71 lines tested in 1949, 46 in 1950, 14 in 1951 and 112 in 1952. The malting test samples numbered 64 in 1950, 23 in 1951, 38 in 1952 and 118 in 1953.

### METHODS

For prediction test, a sample of barley which has been cleaned free from awns, empty hulls, foreign seeds, etc., and weighing about 60 grams, is preferred. A 1000-kernel weight measurement is made by the plant breeder before sending the same to the laboratory.

The barley sample is prepared for analysis by grinding in a Wiley mill, using a 1.0-mm. sieve, and the analytical determinations made are the moisture content, total nitrogen content, barley extract and barley saccharifying activity. Moisture content is measured by drying a 5-gram sample at 115° C. for two hours and total nitrogen content by a modified Kjeldahl method. The barley extract is determined by the revised method of Meredith (6) and the method employed for saccharifying activity is that of Sallans and Anderson (9). As it is some time since the procedures for the determination of the extract and saccharifying activity were published, they are described below.

Predicted extract yield is determined by digesting the barley with a solution containing malt enzymes and measuring the specific gravity of the resulting wort. A sample of 15 grams of ground barley (as is) is weighed into a tared nickel beaker and thoroughly mixed with 100 ml. of an aqueous solution containing 0.2 grams of alpha-amylase and 0.5 grams of malt diastase. The mixture is allowed to stand at 20° C. for 16-18 hours, and then mashed under continuous stirring. The beaker is placed in the mash bath at 48° C. to 50° C. and this temperature maintained for 10 minutes. The temperature is raised at the rate of one degree per minute to 75° C. and maintained at that level for 30 minutes. The bath is then cooled to room temperature in about 10 minutes, the beaker is removed and the



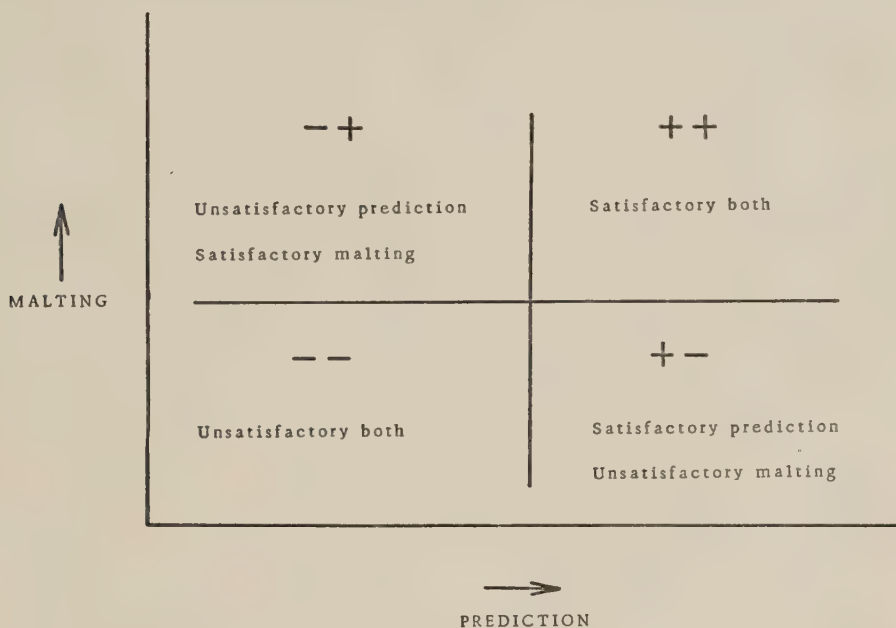


FIGURE 1. Diagram illustrating method of assessment.

weight of the contents made up to 135 grams by the addition of water. The mash is filtered and the specific gravity of the filtrate determined pycnometrically at 20° C. Parallel with this procedure, a blank determination of the specific gravity of the enzyme solution employed is carried out and the specific gravity in excess of one is subtracted from the specific gravity of the barley wort. The extract yield of the barley is determined by reference to the appropriate table in the "Tables for Extract Determination in Malt and Cereals" of the American Society of Brewing Chemists (1).

The method for predicted saccharifying activity consists of activating the barley by means of a solution of papain and then determining the amount of sugar formed by the action of the resulting infusion on starch. A mixture of 2.5 grams of ground barley (as is) and 50 ml. of a one per cent solution of papain is allowed to stand at 20° C. for 20 hours. The mixture is filtered and one ml. of the filtrate is allowed to react for exactly 30 minutes at 20° C. with 100 ml. of a 2 per cent starch solution, buffered to pH 4.6 using an acetate buffer. The reaction is stopped by the addition of 10 ml. of 0.1 N sodium hydroxide and the total volume is made up to 200 ml. The amount of reducing sugars present in a 5 ml. aliquot of the digested starch solution is determined by the ferricyanide procedure as modified by Anderson and Sallans (3). The 5-ml. aliquot, mixed with 10 ml. of 0.05 N alkaline potassium ferricyanide solution, is heated in a water bath at the boiling point, care being taken to avoid evaporation of the reacting mixture. After exactly 20 minutes, the mixture is cooled quickly to 20° C. and to it are added 25 ml. of an acetic acid-salt solution (70 grams potassium chloride, 20 grams zinc sulphate, 200 grams glacial acetic acid, diluted with water to one litre) and then one ml. of 50 per cent

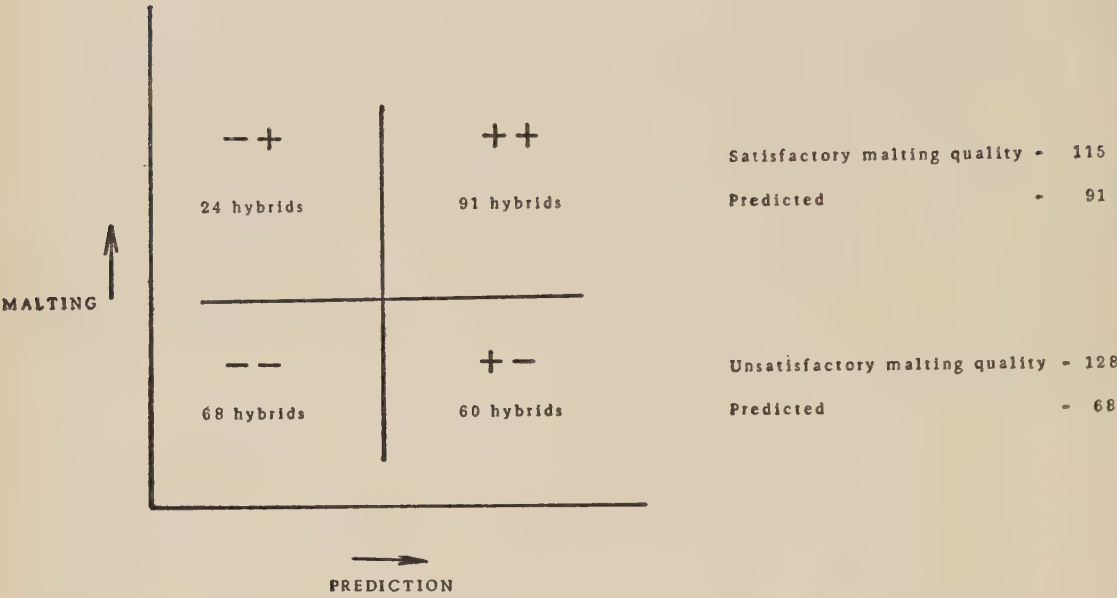


FIGURE 2. Distribution of hybrids according to system illustrated in Figure 1.

potassium iodide solution. The resulting mixture is titrated with 0.05 N sodium thiosulphate until the blue colour just disappears. A blank determination of the reducing power of the starch and the other reagents employed in the analysis is made. The volume of potassium ferricyanide reduced by the sugars of the digested starch solution, less that volume due to the starch, is multiplied by 36 to give the barley saccharifying activity value in degrees Lintner.

RESULTS AND DISCUSSION

The predicted extract and saccharifying activity of each hybrid line from one year's crop and the malt extract and saccharifying activity of the same hybrid line grown in a following year were compared with the data for a standard variety. Using the analytical data, those lines with a predicted extract and predicted saccharifying activity equal to or higher than the standard variety were classed as being satisfactory; and those lines with lower extract or saccharifying activity or both as being unsatisfactory. The same assessments were made using the data from malt analyses. This system of assessment is illustrated in Figure 1.

A diagram was prepared (Figure 2) in which the barleys were separated into the four groups dependent upon the results of prediction and malting tests. The datum lines represent the standard variety. One quadrant contains hybrids satisfactory in both malting and prediction tests, another quadrant is for lines unsatisfactory in both tests and the third and fourth quadrants contains those hybrids which gave different results in each test. Table 1 lists the sets of hybrids examined, the varieties included in their parentage, the number of lines in each quadrant and the total number of lines in each set.

The number of hybrid lines that had appeared promising on the basis of prediction tests totalled 151 (Figure 2). Of these, 91 lines—or 60 per cent—showed good quality when grown later and subjected to malting tests. This is a considerable concentration of good quality material, as normally only a relatively low percentage of hybrid lines from any one station appears satisfactory in the prediction tests. The 60 lines that later were unsatisfactory in the malting tests are a form of insurance against the loss of useful material. The percentage of satisfactory material in the lines recommended for advanced tests represents a level of efficiency that cannot be approached by guesswork. Another measure of the efficiency of the prediction tests is provided by the 92 lines that appeared unsatisfactory by the prediction tests but which were retained by the plant breeder and were also malted. Of these, 68—or 74 per cent—were unsatisfactory on malting. However, the fact that 24 lines malted better than had been predicted is worthy of close examination.

Plant breeders may be quite justifiably disturbed at the thought of rejection of any useful material, even though there is a high proportion of satisfactory hybrids in the group approved by the prediction tests. Such failure could be held against the prediction test, despite its other obvious successes. Of the rejected 24 lines, 5 were faulted for low predicted extract, though the value was on the borderline, and the rest were low in predicted saccharifying activity. On these grounds they were not recommended as possible malting types. Investigation has shown that 18 of the samples faulted for low predicted saccharifying activity had the varieties Peatland and Newal in their parentage. This combination was suspect at the time the prediction test was developed (8). The saccharifying activities of O.A.C. 21  $\times$  Peatland lines tended to be underestimated and those of Newal lines tended to be overestimated. On the basis of the present study the effect of Peatland prevails, and the presence of this variety in the parentage of a hybrid still leads to underestimation of the malt saccharifying activity. It is, therefore, important that the chemist be informed of the parentage of lines submitted for prediction tests in order that the necessary allowances can be made, as a safeguard against premature rejection of a line of real possible value.

### CONCLUSION

The comparison of the results of prediction tests with the results of malting tests on material grown in a following year, as was done in this study, provides an exacting criterion of the reliability of the prediction test as a method of selecting hybrids with potential malting quality. On this basis the present efficiency of the prediction test in use in Canada is 79 per cent, which is approximately the effectiveness forecast when the method was developed some 12 years ago. The continued success of this method depends upon close co-operation between the plant breeder and the malting chemist. It is advisable that the chemist be informed of the parentage of the hybrids he tests, particularly where Peatland occurs in the cross, as there is still danger that the value of these crosses will be underestimated by the prediction method. Peatland has been used widely as a

source of rust resistance and thus this anomalous behaviour of Peatland hybrids is an important factor to be considered. Newal does not present a serious problem as it merely adds to the material to be malted and which will be rejected later.

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# SEED TREATMENT OF FORAGE LEGUMES AND GRASSES WITH THREE ANTIBIOTICS

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## ABSTRACT

Aureomycin, penicillin and terramycin were applied as seed treatments at several concentrations to five forage species. All three antibiotics stimulated seedling growth markedly in alfalfa, moderately in red clover and not at all in timothy in greenhouse trials. No stimulation was detected in field trials with alfalfa, red clover, brome grass and reed canary grass. The antibiotics had no effect on the number of seedlings established except in brome grass where small variable differences were obtained. The results of this study do not justify recommendation of seed treatment of these five forage species with the antibiotics used.

Farmers in several sections of Ontario are experiencing difficulty in obtaining satisfactory stands of forage legumes and grasses. For example, forage seedling establishment is rated the most important crop production problem in Haldimand county. Since several references in the literature have indicated a response of germinating seeds to antibiotics, a study was undertaken to evaluate three antibiotics as seed treatments considering their effect on number of seedlings established and seedling vigour. No attempt was made to obtain information regarding the manner in which these antibiotics act in affecting plant growth.

Barton and MacNab (1) summarized the recent literature on the effect of antibiotics on plant growth. Few studies have been reported using forage plants. Euler (2) found that several antibiotics depressed growth of the primary leaves in timothy and perennial rye-grass seedlings grown in the laboratory. Severe reduction in the root development of red clover treated with 13 antibiotics was reported by Wright (5). The degree of the reduction varied with the antibiotic used and with the concentration. No antibiotic produced an appreciable amount of stimulation. Gregory *et al.* (3) from greenhouse studies, concluded that both antibiotics and antagonistic micro-organisms show promise in the control of damping-off in alfalfa caused by *Pythium* spp. Of the six tested, none was satisfactory and two caused severe stunting in the alfalfa seedlings. Hervey (4) found increases in germination and plant vigour in sweet clover and increases in germination in buffelgrass *Pennisetum ciliare* (L.) Link, treated with four antibiotics in greenhouse studies.

## MATERIALS AND METHODS

Aureomycin, penicillin and terramycin were applied as seed treatments to alfalfa, red clover, timothy, brome grass and reed canary grass in greenhouse and field studies. The antibiotics were applied at the rate of 1/32, 1/8, 1/2 and 2 grams per bushel of seed. Solutions or suspensions of the antibiotic were made, using distilled water and diluted so that one cc. of the

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material on one ounce of seed gave the required treatment application. After treatment the seed was dried, and planted immediately in all greenhouse studies and planted the following day in the field trials.

Greenhouse studies were conducted with alfalfa, red clover and timothy in 1953 using Haldimand clay soil. The alfalfa series was replanted on a Guelph loam soil in 1954. An experimental unit in the greenhouse consisted of 100 seeds planted at a depth of  $\frac{3}{4}$  inch in a 4-inch clay pot. In 1953 alfalfa, red clover, bromegrass and reed canary grass were grown in field trials at Guelph. In these studies, 100 seeds were placed at a depth of  $\frac{3}{4}$  inch in a 2 $\frac{1}{2}$ -foot row. The broadcast plantings in 1953 of alfalfa and red clover were seeded in plots 5 feet  $\times$  22 feet at the rates of 12 lb. of alfalfa and 10 lb. of red clover seed per acre. Four replications were used in all trials. Plant stands were taken at 2 to 4 weeks from planting. Vigour indices by scores, plant weights or heights were recorded at 10 to 16 weeks after seeding. The data on stand counts were transformed for analysis but the actual percentage data are reported.

## RESULTS AND DISCUSSION

### *Alfalfa*

Treatment with antibiotics had no effect on number of seedlings established as shown in Tables 1 and 2. Striking effects were observed in seedling vigour in both greenhouse trials. Figure 1 shows the increase in vigour resulting from treatment in 1953, and Table 1 lists the height data from the 1954 greenhouse trial. All three antibiotics were effective in

TABLE 1.—RESPONSE OF ALFALFA TO SEED TREATMENT WITH ANTIBIOTICS IN GREENHOUSE STUDIES

Grams of antibiotic per bushel of seed	Per cent stand*		Seedling height at 16 weeks, in cm.
	1953	1954	1954
Aureomycin 1/32	60.9	59.5	15.5
$\frac{1}{8}$	58.2	60.7	24.1
$\frac{1}{2}$	63.8	69.4	24.5
2	60.1	59.7	19.6
Terramycin 1/32	63.3	61.7	15.0
$\frac{1}{8}$	65.8	64.3	24.1
$\frac{1}{2}$	62.3	62.6	24.6
2	56.1	66.1	18.6
Penicillin 1/32	58.8	69.0	10.6
$\frac{1}{8}$	57.0	56.6	16.9
$\frac{1}{2}$	62.3	64.1	18.6
2	65.3	68.7	11.6
Check	61.4	70.8	10.6
L.S.D. P. 0.05	N.S.	N.S.	3.2
P. 0.01	—	—	4.3
C.V.	10.0	10.5	12.4

\* Stand at 8 and 6 weeks in 1953 and 1954, respectively.



FIGURE 1. Comparison of the response of alfalfa and red clover to seed treatment with antibiotics at 10 weeks from seeding in 1953. The most effective rate in each antibiotic is shown along with the check.

*Top:* Alfalfa, *left to right*, check, aureomycin 1/2, penicillin 1/2, and terramycin 1/8 gm.

*Bottom:* Red clover, *left to right*, check, aureomycin 1/2, penicillin 1/8, and terramycin 1/32 gm.



FIGURE 2. Effect of aureomycin (*top*), terramycin (*middle*) and penicillin (*bottom*) on vigour of alfalfa seedlings at 10 weeks from seeding in 1953. From left to right in each group, check, 1/32, 1/8, 1/2 and 2 gm. antibiotic.



TABLE 2.—RESPONSE OF ALFALFA TO SEED TREATMENTS WITH ANTIBIOTICS  
IN FIELD PLANTINGS AT GUELPH IN 1953

Grams of antibiotic per bushel of seed	Row plantings			Broadcast planting, May	
	May seeding per cent stand at 5 weeks	August seeding per cent stand at 4 weeks	Vigour*	No. plants per sq. foot at 5 weeks	Dry weight 25 plants at 8 weeks
Aureomycin 1/32	29.7	47.4	2.0	25.4	74.2
1/8	21.0	42.2	2.2		
1/2	27.6	46.2	2.8		
2	31.7	39.4	3.5		
Terramycin 1/32	24.6	42.9	2.8	28.8	79.2
1/8	37.1	40.3	2.5		
1/2	30.1	47.2	2.8		
2	31.3	46.0	2.2		
Penicillin 1/32	35.5	33.5	3.2	24.9	84.0
1/8	28.4	46.2	3.0		
1/2	25.1	45.8	1.5		
2	25.3	44.4	2.5		
Check	27.6	47.4	1.8	24.0	82.5
Significance of F value	N.S.	N.S.	N.S.	N.S.	N.S.
C.V.	17.5	12.2	32.2	8.3	10.7

\* Vigour index 1 (*good*) to 5 (*poor*) at 9 weeks.

increasing vigour in both trials and in each case aureomycin and terramycin were equally effective. Penicillin was nearly as effective as these in the 1953 trial but only one-half as effective in increasing vigour in the 1954 study. In 1954 the mean seedling height for check, aureomycin, terramycin and penicillin was 10.6, 21.9, 20.6 and 14.9 cm., respectively.

In both years the medium concentrations gave the greatest response as indicated in Figure 2 and Table 1. No phytotoxic effects were observed at the high rates of application. Although the stimulation of seedling growth was striking in the greenhouse no such effects were detected in the three field trials.

### Red Clover

The data in Table 3 indicate that the antibiotics did not affect plant stands. An increase in seedling vigour was observed in the greenhouse trial but, as shown in Figure 1, the increase was not as marked as with alfalfa. The response was similar at the 1/32-, 1/8- and 1/2-gram rates and superior to the check. At the 2-gm. rate the seedling vigour was similar to that in the check for terramycin and penicillin and slightly better than the check for aureomycin. In a subsequent test, using rates of 4 and 8 grams of each antibiotic, a depressing effect on growth was observed. In most cases the leaves were smaller, curled and slightly chlorotic, but plant stands were not reduced. No effects on plant stand or vigour were found in the field plantings.

TABLE 3.—RESPONSE OF RED CLOVER TO SEED TREATMENT WITH ANTIBIOTICS IN GREENHOUSE AND FIELD TRIALS AT GUELPH IN 1953

Grams of antibiotic per bushel of seed	Greenhouse	Field row planting			Field broadcast planting	
	Per cent stand at 8 weeks	May seeding	August seeding		Plants per square foot at 5 weeks	Dry weight of 25 plants at 8 weeks
		Per cent stand*	Per cent stand*	Vigour**		
Aureomycin 1/32	60.4	33.5	15.1	3.8	39.7	55.2
1/8	64.3	35.0	15.5	2.8		
1/4	60.7	39.3	18.7	3.0		
2	63.8	41.8	26.4	2.8		
Terramycin 1/32	64.3	44.6	14.3	3.5	35.2	56.2
1/8	65.1	44.4	26.7	2.5		
1/4	65.1	43.7	25.8	2.5		
2	64.5	38.4	20.1	3.2		
Penicillin 1/32	60.1	37.6	20.9	3.0	39.7	55.0
1/8	57.3	35.0	19.4	2.8		
1/4	57.1	53.0	25.8	3.2		
2	70.2	43.9	23.2	3.0		
Check	61.6	38.1	13.3	3.2	37.2	57.8
Significance of F value	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
C.V.	6.1	12.4	20.1	32.1	18.0	14.9

\* Stand counts at 5 and 4 weeks for May and August seeding, respectively.

\*\* Vigour index 1 (*good*) to 5 (*poor*) at 9 weeks.

TABLE 4.—RESPONSE OF GRASSES TO SEED TREATMENT WITH ANTIBIOTICS IN GREENHOUSE AND FIELD PLANTINGS AT GUELPH IN 1953

Grams of antibiotic per bushel of seed	Greenhouse	Field row plantings*					
	Timothy	Bromegrass I		Bromegrass II		Reed canary grass	
	Per cent stand at 7 weeks	Per cent stand	Dry weight 25 plants	Per cent stand	Dry weight 25 plants	Per cent stand	Dry weight 25 plants
Aureomycin 1/32	94.5	68.9	6.4	55.6	4.8	51.7	6.0
1/8	90.2	62.9	6.0	52.4	6.4	49.7	5.1
1/4	93.2	61.6	8.7	55.2	6.4	47.7	7.0
2	90.2	60.0	7.7	46.3	5.2	50.5	5.4
4	—	67.4	6.1	55.6	5.2	47.6	5.7
Terramycin 1/32	92.2	48.6	7.5	61.6	5.3	44.4	5.6
1/8	89.6	57.9	6.4	61.6	4.7	51.4	5.5
1/4	87.6	61.6	7.6	54.4	5.1	47.2	5.9
2	83.1	55.7	6.5	44.4	4.9	39.4	5.8
4	—	48.6	6.6	44.6	4.8	47.4	5.9
Penicillin 1/32	92.6	67.1	6.3	61.7	6.3	51.4	5.4
1/8	80.1	65.9	8.0	49.3	5.9	49.5	5.3
1/4	89.6	61.1	6.7	52.3	5.3	46.0	6.0
2	90.8	59.9	9.0	49.6	5.2	43.9	8.1
4	—	67.3	7.3	58.3	5.4	52.6	6.6
Check	93.4	66.1	7.5	47.7	5.4	50.5	5.5
L.S.D. 0.05	N.S.	Sig.**	N.S.	Sig.**	N.S.	N.S.	N.S.
C.V.	8.2	9.4	21.6	10.4	22.2	14.4	24.1

\* Stand counts at 2 weeks and dry weight measurements at 6 weeks.

\*\* L.S.D. not listed because it applies only to transformed data which are not shown.

*Grasses*

The results of studies with grasses are summarized in Table 4. Antibiotic seed treatments had no effect on the stand or seedling vigour of timothy grown in the greenhouse in 1953. Since more difficulty is experienced in obtaining satisfactory stands of brome grass and reed canary grass, these grasses were used in the field trials. In addition one higher rate, 4 grams per bushel, was included to widen the range of concentrations tested. In general, there was no response to treatment, although differences were found in plant counts in each of the two brome grass tests. These differences were variable and of questionable significance. In the first trial, the 1/32- and 4-gram rates in terramycin gave lower stands than the check but no treatment increased stands. In the second trial, three treatments, terramycin 1/32 and 1/8 and penicillin 1/32, gave higher stands than the check, while stand counts were similar in other treatments.

Vigour differences were not detected in the three field trials.

Although striking differences were observed with the legumes in greenhouse trials, no marked response was found in the field studies. Consequently the results of this study do not justify recommendation of seed treatment of these forage species with the antibiotics used.

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# FERTILIZER STUDIES WITH RADIOACTIVE SULPHUR. II,<sup>1</sup>

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## ABSTRACT

Sulphur fertilization studies with grain and legume crops at 22 Alberta locations, 1950-1952, are reported.  $\text{Na}_2\text{SO}_4$  containing  $\text{S}^{35}$  as well as several different sulphur containing fertilizers were used.

Sulphur deficiency is found to be erratic but important in Alberta's grey soils zone. The materials analysed were generally low in this element compared to similar materials from other regions. Previous plot history and current fertilization both strongly affected the sulphur content of crops, especially of grain straws. Large proportions of the sulphur in crops came from fertilizer applied broadcast and considerable amounts were taken up from placements at 10- and 22-inch depths. No evidences of radiation injury from  $\text{S}^{35}$  were found.

## INTRODUCTION

Sulphur deficiency in some Alberta grey wooded soils and the use of  $\text{S}^{35}$  as a tracer in fertilizer studies have been described (2). Because of the great importance of suitable fertilizers on these grey soils it is important to ascertain the extent and location of the areas and to determine the most suitable kinds and amounts of fertilizers for use on them. A means of determining sulphur deficiency in soils by means of chemical tests rather than by field fertilizer trials is sought. This report is concerned with a continuation of investigations begun in 1949 (2) and deals with field and laboratory studies conducted in 1950, 1951, and 1952.

## MATERIALS AND METHODS

The experiments were conducted on legumes and grains at Breton Experimental Field, 75 miles southwest of Edmonton, at the Soils Department Plots, Edmonton, and on farm fields in widely separated areas. The locations, shown in Figure 1, were unevenly scattered because selection of fields was determined by soil, crop, accessibility and owner interest. Alfalfa was the legume on farm fields with the exceptions of locations 4 and 10 where alsike was grown. The experiments are grouped and outlined in the following sections:

### I. Sulphur-Uptake Experiments with Radioactive Sulphur as a Tracer

Small volumes of chemically pure  $\text{Na}_2\text{SO}_4$  solutions containing approximately 0.01 mc.  $\text{S}^{35}$  per gram of  $\text{Na}_2\text{SO}_4$  were applied to grains and legumes at various locations and rates. Details are given in Table 1.

The Breton Plots, on Breton loam (1), have been operated by the Soils Department, University of Alberta, since 1930. A severe sulphur deficiency on the area and on farm lands in the vicinity has been clearly established.

<sup>1</sup> Contribution from Department of Soil Science and Department of Physics, University of Alberta.

<sup>2</sup> Associate Professor of Soil Science; former graduate student (now graduate student, Ohio State College), and Professor of Physics, respectively.



TABLE 1.—SULPHUR-UPTAKE EXPERIMENTS USING RADIOACTIVE SULPHUR

Place and year	Crops	Total area treated with $\text{Na}_2\text{S}^*\text{O}_4$	Area per sample	Number of samples per treatment
1. <i>On longtime experimental plots—</i> A. Breton Plots. 1950	Alfalfa, altaswede clover, grains	1 sq. yd. $\times$ 6 5 ft. $\times$ 17 ft.	4 sq. ft. 1 sq. yd.	6 5
Breton Plots. 1951 and 1952	Grains	5 ft. $\times$ 22 ft.	1 sq. yd.	6
B. Soils Plots, Edmonton. 1951 and 1952	Legumes and grains	25 sq. ft. $\times$ 4 5 ft. $\times$ 22 ft.	1 sq. yd. 1 sq. yd.	4 6
2. <i>On farm fields,</i> 1951 and 1952	Legumes Grains			

Samples were taken from the central portion of the treated areas and each sample had a border of treated area on all sides of it.

The Soils Department Plots at Edmonton, located on Malmo clay loam (1), were established in 1931 and there is no sulphur deficiency in the area nor on farm lands in the vicinity.

Only three of the farm fields were in areas where the soil series has been established. Information and analytical data for these as well as for Experimental Plot soils are tabulated in Table 2.

TABLE 2.—ANALYTICAL DATA AND CLASSIFICATION INFORMATION FOR SOILS

Location	Approx. years cultivated	S per cent surface 6-in.	pH	Parent material
<i>Locations not definitely responsive to sulphur fertilization of legumes</i>				
2	40	0.067	5.9	Lacustrine <sup>1</sup>
5	8	0.015	6.0	Glacial till
7	40	0.028	6.5	Lacustrine
8	15	—	6.7	Lacustrine
9	12	—	7.1	Lacustrine
14	15	0.025	6.6	Glacial till
15	20	0.014	6.6	Glacial till and residual
<i>Locations definitely responsive to sulphur fertilization of legumes</i>				
1	30	0.010	6.5	Glacial till <sup>2</sup>
3	2	0.018	6.4	Glacial till <sup>3</sup>
4	15	0.013	6.6	Glacial till
6	20	0.018	5.8	Lacustrine
10	14	—	6.5	Glacial till and residual
11	10	0.019	6.9	Glacial till
12	10	—	—	Glacial till
13	23	0.018	6.2	Glacial till
<i>Locations where grains only were grown</i>				
16	20	0.011	6.6	Glacial till and residual
17	26	0.013	5.9	Glacial till and residual
18	1	0.009	6.3	Glacial till
19	20	0.019	6.7	Glacial till
20	25	0.009	6.9	Glacial till
21	25	0.013	6.7	Glacial till
22	55	0.040	6.1	Lacustrine <sup>4</sup>

<sup>1</sup> Soils Dept. plots, Edmonton, Malmo clay loam (1).

<sup>2</sup> Breton plots, Breton loam (1).

<sup>3</sup> Breton loam.

<sup>4</sup> Malmo clay loam.

NOTE: Locations 2 and 22 have black zone soils and Location 6 has degraded black soil. All other locations have grey wooded soils.

For experiments with legumes, the  $\text{Na}_2\text{S}^*\text{O}_4$  <sup>1</sup> solutions were applied as early in the spring as convenient. For grains, applications were made after seeding but before the crop was up. At Edmonton and Breton, treatments were made near the ends of the established plots. The treated

<sup>1</sup>  $\text{Na}_2\text{S}^*\text{O}_4$  denotes sodium sulphate containing  $\text{S}^{35}$  as a radioactive tracer.

TABLE 3.—YIELD, NITROGEN AND SULPHUR CONTENT OF LEGUMES GROWN AT BRETON PLOTS WITH  $\text{Na}_2\text{S}^*\text{O}_4$ 

Plot history and $\text{Na}_2\text{S}^*\text{O}_4$ treatment	Alfalfa			Atlaswede clover			
	Yield, gm. per sq. yd.	N per cent	S per cent	S per cent from fertilizer	Yield, gm. per sq. yd.	N per cent	S per cent from fertilizer
$(\text{NH}_4)_2\text{SO}_4$ since 1930 <sup>1</sup>							
Amt. $\text{Na}_2\text{S}^*\text{O}_4$ applied 1950							
0	19.2	1.85	0.20	0.5	51.7	2.58	0.12
30 lb. per acre	18.2	2.01	0.24	7	60.5	2.41	0.13
100 lb. per acre	14.8	1.93	0.30	10	57.0	2.53	0.14
300 lb. per acre	12.7	1.68	0.30	19	60.0	2.45	0.13
No fertilizer since 1930							
Amt. $\text{Na}_2\text{S}^*\text{O}_4$ applied 1950							
0	6.3	—	0.12	0.4	6.5	2.48	0.12
100 lb. per acre	12.2 <sup>2</sup>	2.15	0.17	68	17.3 <sup>2</sup>	2.76	0.12

<sup>1</sup> 1,200 lb.  $(\text{NH}_4)_2\text{SO}_4$  applied since 1930.<sup>2</sup> Statistically significant yield increase—1 per cent level.

TABLE 4.—YIELD AND SULPHUR CONTENT OF GRAINS GROWN AT BRETON PLOTS WITH AND WITHOUT  $\text{Na}_2\text{S}^*\text{O}_4$ 

Crop and past fertilizer treatments	Total yield, gm./sq. yd.			S per cent				S per cent from fertilizer
	Year	Check	Na <sub>2</sub> S*O <sub>4</sub>	Check		Na <sub>2</sub> S*O <sub>4</sub>		
				Grain	Straw	Grain	Straw	
Wheat, continuously since 1930 NS since 1930 NPKS since 1930	1950	65	99 <sup>1</sup>	0.15	0.17	0.14	0.19	24
	1952	173	187	0.11		0.12		28
	1950	97	124	0.14	0.09	0.13	0.15	42
	1952	68	128 <sup>1</sup>	0.11		0.14		53
Wheat, 5-year rotation since 1930 NS since 1930 NPKS since 1930	1950	142	230 <sup>2</sup>	0.17	0.13	0.18	0.15	18
	1952	212	306 <sup>1</sup>	0.11		0.14		32
	1950	38	169 <sup>2</sup>	0.14	0.05	0.13	0.12	62
	1952	116	194 <sup>1</sup>	0.10		0.11		60
Oats, 5-year rotation since 1930 NS since 1930 NPS since 1930	1950	119	108	0.18	0.14	0.19	0.19	28
	1952	67	113 <sup>1</sup>	0.16		0.16		40
	1950	47	95 <sup>2</sup>	0.14	0.04	0.18	0.16	50
	1952	65	95	0.13		0.14		57
Barley, 5-year rotation since 1930 NS since 1930 NPS since 1930	1950	45	50	0.18	0.12	0.18	0.13	26
	1952	78	109 <sup>1</sup>	0.14		0.14		38
	1950	11	27 <sup>2</sup>	0.15	0.03	0.17	0.15	37
	1952	42	68	0.08		0.11		64

<sup>1</sup> Statistically significant yield increase—5 per cent level.<sup>2</sup> Statistically significant yield increase—1 per cent level.

## NOTES:

(a) "Wheat, continuously since 1930". This area has been cropped to wheat only, since 1930.

(b) "5-year rotation since 1930". This area has been cropped to the following rotation since 1930: wheat, oats, barley plus legumes seeded, 2 years legume hay.

(c) "NS" plots: 1,200 lb.  $(\text{NH}_4)_2\text{SO}_4$  per acre (300 lb. S) since 1930. "NPKS" plots: 1,000 lb. ammonium phosphate 16-20-0 and 450 lb.  $\text{K}_2\text{SO}_4$  per acre (300 lb. S) since 1930. "NPS" plots: 1,000 lb. ammonium phosphate 16-20-0 per acre (140 lb. S) since 1930.

(d) The "check" samples of 1950 contained 0.8-1.7 per cent of fertilizer sulphur. 1952 check samples contained no radioactive sulphur.

(e)  $\text{Na}_2\text{S}^*\text{O}_4$  at 100 lb. per acre.



and check areas were parallel and samples were harvested on a paired basis. On farm fields, legume treatments were replicated and randomized while for grains, strips were treated and check samples were taken on a paired basis from a parallel strip 5 feet away.

In 1949 check samples obtained within 6 inches of the treated plots contained appreciable amounts of  $S^{35}$  (2). A few 1950 samples taken over 1 foot from treated areas had a low content of fertilizer sulphur. During 1951 and 1952 at least 5 feet separated checks from  $Na_2S^*O_4$  treated areas. No  $S^{35}$  was found in these samples with two exceptions. In those two cases downpours occurred soon after the fertilizer applications were made and contamination is attributed to surface movement of water.

In order to determine the extent of  $S^{35}$  uptake due to lateral movement of roots and  $Na_2S^*O_4$ , samples were taken parallel to treated plots 6, 12, and 24 inches from them on four farms in 1951.

## II. Yield Experiments with Sulphur-Containing Fertilizers

Various sulphur-containing fertilizers were applied to farm fields of legumes and grains in conjunction with the Sulphur-Uptake Experiments. The treatments and rates of application were as follows:

Fertilizer treatment	Pound per acre applied	Pound of sulphur per acre supplied
None (check)	—	—
Flowers of sulphur	10	10
Flowers of sulphur	20	20
Flowers of sulphur	50	50
$CaSO_4$ (gypsum)	111	20
$(NH_4)_2SO_4$	80	20
$Na_2SO_4$	91	20
Ammonium phosphate 16-20-0	143	20
$Na_2SO_4$ plus trace element mixture*	68	20
	50	

\* The trace element mixture consisted of the following materials and ratios:  $MnSO_4$ , 30 lb.;  $CuSO_4$ ,  $ZnSO_4$ , and borax ( $Na_2B_4O_7$ ), 15 lb. each;  $Na_2MoO_4 \cdot 2H_2O$ , 1 lb.

In the 1951 experiments individual plots were 40 feet by 40 feet and each treatment was replicated four times. In the 1952 experiments plots were 20 feet by 20 feet and each treatment was replicated six times. Yield data were obtained by harvest of two 1 square-yard samples selected at random in each plot.

TABLE 5.—YIELD AND SULPHUR CONTENT OF GRAINS AND LEGUMES GROWN ON UNIVERSITY OF ALBERTA SOILS DEPT. PLOTS, EDMONTON, WITH AND WITHOUT  $\text{Na}_2\text{S}^*\text{O}_4$

Crop and past fertilizer treatments	Total yield, gm./sq. yd.			S per cent					S per cent from fertilizer
	Year	Check	Na <sub>2</sub> S*O <sub>4</sub>	Check		Na <sub>2</sub> S*O <sub>4</sub>			
				Grain	Straw	Grain	Straw		
Wheat, NS since 1931 NS since 1931	1951	284	346	0.17	0.16	0.15	0.21	26	
	1952	168	170	0.16		0.16		19	
No fertilizer since 1931 No fertilizer since 1931	1951	245	302	0.16	0.10	0.13	0.16	33	
	1952	172	157	0.16		0.14		27	
Barley, NS since 1931 NS since 1931	1951	264	239	0.16	0.18	0.16	0.22	33	
	1952	132	144	0.15		0.15		24	
No fertilizer since 1931 No fertilizer since 1931	1951	214	194	0.14	0.11	0.16	0.19	46	
	1952	129	127	0.14		0.15		41	
Alfalfa, No fertilizer since 1931	1951	—	—	—	0.13	—	0.20	30	
Sweet Clover, NS since 1931 No fertilizer since 1931	1952	—	—	—	0.28	—	0.30	34	
	1952	—	—	—	0.20	—	0.26	48	
Atlaswede Clover, NS since 1931 No fertilizer since 1931	1952	—	—	—	0.17	—	0.16	28	
	1952	—	—	—	0.16	—	0.18	35	

NOTES (a) "NS" plots: 1,000 lb.  $(\text{NH}_4)_2\text{SO}_4$  per acre (250 lb. S) since 1931.  
 (b) None of the check samples contained any radioactive sulphur.  
 (c)  $\text{Na}_2\text{S}^*\text{O}_4$  at 100 lb. per acre.

### III. Feeding Depth Experiments

In order to determine the extent of sulphur uptake from lower soil horizons, small volumes of  $\text{Na}_2\text{S}^*\text{O}_4$  and  $\text{Na}_2\text{SO}_4$  solutions were placed at 10- and 22-inch depths in two established stands of alfalfa and in one grain field. The treatments, replicated four times, were applied to plots one square yard in area. These experiments were associated with Sulphur-Uptake Experiments. The  $\text{Na}_2\text{S}^*\text{O}_4$  solution was placed at the depths indicated by pouring the solution through a glass tube into holes punched with a pointed steel rod 0.5 inch in diameter. Twelve uniformly spaced holes were made in each treated square yard area and were filled with sand after the treatments were made. Samples were taken from the central 4 square feet.

#### *Sampling*

For all types of experiment the yield and analytical samples were hand harvested. All samples for analysis were carefully selected complete plants free of weeds and other extraneous materials. In the case of plant mixtures, such as the alfalfa and Altaswede clover at Breton, analytical materials were obtained by harvesting individually a sufficient number of whole plants to ensure pure samples of each species.

The soil samples represented a composite of the surface six inches. Samples at the Breton and Edmonton Experimental plots were taken from areas which had not been fertilized since 1930.

#### *Analytical Methods*

Where  $\text{S}^{35}$  determinations were made, approximately 12 grams of legume and grain samples and 20 grams of straw were ashed by the nitric and perchloric acids procedure (6). Total S and  $\text{S}^{35}$  contents were determined by precipitation as  $\text{BaSO}_4$  according to the procedure described by Cormack *et al.* (2).

For other sulphur determinations on plant materials, duplicate two-gram samples were ashed and sulphur was determined turbidimetrically using a Beckman Model DU Spectrophotometer. Solutions to be analysed were diluted to contain 10-100 p.p.m. of  $\text{SO}_4^{=}$  and to be 0.1 N with HCl. Approximate photometer settings were: wave length 5000Å, slit width 0.15 mm., selector switch 1.0 position, red-sensitive photo tube.

Before use in these experiments the turbidimetric method of sulphur determination was carefully compared with the gravimetric method. Twenty paired comparisons did not reveal any difference in the reliability of the two methods. Standard deviations of less than 0.005 per cent sulphur were found for both methods.

The Kjeldahl-Gunning method (9) modified by use of selenium as a catalyst was used for nitrogen determination.

Composite samples of plant materials were prepared by taking equal portions from all replicates of each treatment. Determinations were made in duplicate. All analytical data are based on oven-dry samples.

The turbidimetric method was used for total sulphur determination in soil samples which had been fused with sodium carbonate (9).

TABLE 6.—YIELD, NITROGEN AND SULPHUR CONTENT OF LEGUMES GROWN ON FARM FIELDS WITH AND WITHOUT  $\text{Na}_2\text{S}^*\text{O}_4$ , 1951 AND 1952

Treatment lb./ac. of S as $\text{Na}_2\text{S}^*\text{O}_4$	Yield, ton/acre		N per cent		S per cent		S per cent from fertilizer	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
<i>Locations not definitely responsive to sulphur fertilization. (No. 4, 5, 7, 9, and 15. Figure 1)</i>								
0	1.1 (5) <sup>1</sup>	0.8 (4)	3.00 (5)	2.51 (4)	0.19 (5)	0.16 (4)	0	0
10	1.3 (5)	1.2 (4)	3.12 (5)	2.51 (4)	0.23 (5)	0.16 (4)	39 (5)	29 (4)
20	1.3 (5)	1.2 (4)	3.25 (5)	2.58 (4)	0.27 (5)	0.18 (4)	51 (5)	42 (4)
50	1.3 (5)	1.2 (4)	3.16 (5)	2.50 (4)	0.29 (5)	0.19 (4)	57 (5)	51 (4)
<i>Locations definitely responsive to sulphur fertilization. (No. 3, 4, 6, 10, 11, 12, and 13. Figure 1)</i>								
0	0.8 (6)	0.6 (6)	2.91 (7)	2.61 (7)	0.15 (7)	0.12 (7)	0.1 (7)	0.02 (7)
10	1.1 (6)	0.9 (6)	3.18 (7)	2.70 (7)	0.23 (7)	0.16 (7)	56 (7)	42 (7)
20	1.2 (6)	1.0 (6)	3.23 (7)	2.79 (7)	0.25 (7)	0.18 (7)	65 (7)	55 (7)
50	1.1 (6)	1.0 (6)	3.30 (7)	2.82 (7)	0.30 (7)	0.20 (7)	76 (7)	68 (7)

<sup>1</sup> Data are averages for the number of locations indicated by the figures in parentheses.



## ANALYTICAL RESULTS AND DISCUSSION

*Soil Analyses*

Although the analytical data for the soils are incomplete (Table 2) they merit comment. The soils are highly variable in total sulphur content. Despite a tendency for a lower amount of this element in soils from fields responding to fertilization, the total content does not appear to be a suitable means of determining responsiveness to sulphur fertilization. The average sulphur content of the soils responding to fertilization here is lower than the average of 0.02 per cent reported by Dean (3) for grey wooded soils of the Prairie Provinces. Lyon *et al.* (8) list the average sulphur content of humid region soils as 0.04 per cent which is higher than all but two of the soils in Table 2.

*Sulphur-Uptake Experiments with Radioactive Sulphur*

The data for these experiments are presented in Tables 3, 4, 5, 6, and 7.

The data of Table 3 show that, for legumes, previous fertilizer history of the plots has affected yield results and uptake of fertilizer sulphur. The data confirm those previously reported (2) although the percentages of sulphur which came from applied  $\text{Na}_2\text{SO}_4^*$  are somewhat lower here. This is probably due to differences in the growing seasons concerned. Unfavourable growing seasons resulted in such poor legume stands that experiments could not be placed on legumes at Breton Plots in 1951 and 1952.

The yield data for grains at Breton in 1950 and 1952 (Table 4) show that in a majority of the plots, application of  $\text{Na}_2\text{S}^*\text{O}_4$  resulted in significant yield increases. The results are contrary to previous Alberta experience with sulphur fertilization of grain (2, 11) and are unexplained. Two items add to the perplexity of the data. The yield increases are not related to the fertilizer history of the plots since 1930 nor to the per cent of plant sulphur which came from the applied  $\text{Na}_2\text{SO}_4^*$ . Fertilizer history of the plots and the application of  $\text{Na}_2\text{S}^*\text{O}_4$  had very little effect on the amount of sulphur in grain but had a marked effect on the amount in straw. Straw from previously unfertilized plots contained, on the average, less than half as much sulphur as that from the previously fertilized plots. Application of  $\text{Na}_2\text{S}^*\text{O}_4$  more than doubled the amount in straw on the previously unfertilized plots. The amount of fertilizer sulphur in plants was strongly affected by past fertilizer history.

At the Soils Department Plots, Edmonton, no yield increases resulted from applications of  $\text{Na}_2\text{S}^*\text{O}_4$  to grains and legumes although substantial amounts of fertilizer sulphur were taken up by the plants (Table 5). The sulphur content of straws was related to fertilizer history of the plots and to application of  $\text{Na}_2\text{S}^*\text{O}_4$ . The effects are similar to those at Breton. Applications of  $\text{Na}_2\text{S}^*\text{O}_4$  increased the sulphur content of legume hays somewhat less distinctly at the Edmonton Plots.

Data for legumes on farm fields are arranged in two groups in Table 6. Although the two groups show no really sharp differences, a higher percentage of fertilizer sulphur was present in hays from farms where definite yield increases resulted from fertilization. First-cut hays had higher nitrogen and sulphur contents than second-cut hays. Hays from

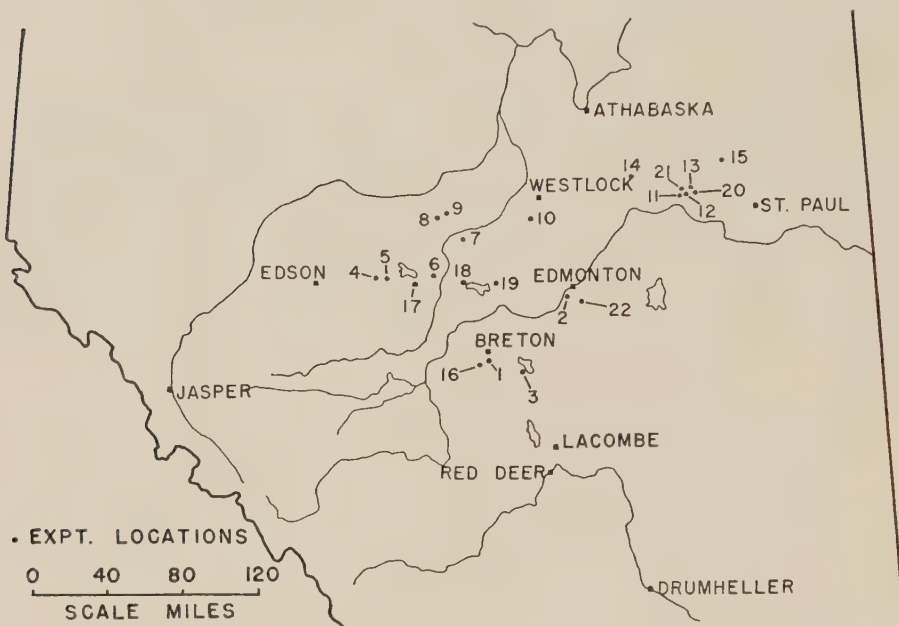


FIGURE 1. Map of Central Alberta with experimental locations indicated by dots and identified by numbers. Information concerning soils at these locations is given in Table 2.

fields responding to fertilization had, on the average, a slightly higher nitrogen content and the per cent of nitrogen increased with increases in amounts of sulphur applied. These hays showed a relatively greater increase in sulphur content as the amount of sulphur applied was increased.

On individual farm fields there was no case where application of  $\text{Na}_2\text{S}^*\text{O}_4$  supplying 20 lb. of sulphur per acre resulted in a statistically significant yield increase of grain crops (Table 7). However, using the data from individual farms as pairs, a *t*-test comparison of data for all the tests on grains shows that the average yield from plots treated with  $\text{Na}_2\text{S}^*\text{O}_4$  was significantly higher than the check plot yield. The nitrogen content of the grains was not altered by the  $\text{Na}_2\text{S}^*\text{O}_4$  treatment. Again fertilized and unfertilized grains show almost no difference in their sulphur content. For samples analysed, application of  $\text{Na}_2\text{S}^*\text{O}_4$  resulted in 100 per cent or more increase in the amount of this element in straw. The proportion of fertilizer sulphur in the farm field grains was rather high, averaging 50 per cent. Sulphur in  $\text{Na}_2\text{S}^*\text{O}_4$  placed at a depth of 10 inches was used in considerable amounts by the grain crops. However, surface applications of the same amount of this material resulted in much greater uptake.

Table 8 established that with readily soluble materials there may be uptake of fertilizer sulphur by crops as far as 2 feet from treated areas during the season of application. However, it would appear unlikely that plant samples taken 5 or more feet from fertilized plots would contain appreciable amounts of fertilizer sulphur.

TABLE 7.—YIELD, NITROGEN AND SULPHUR CONTENT OF GRAINS GROWN ON FARM FIELDS WITH AND WITHOUT  $\text{Na}_2\text{S}^*\text{O}_4$ , 1951 AND 1952

Crop	Year	Yield gm./sq. yd.		N per cent in grain		S per cent				S per cent from fertilizer
		Check	Na <sub>2</sub> S*O <sub>4</sub> <sup>1</sup>	Check	Na <sub>2</sub> S*O <sub>4</sub>	Check		Na <sub>2</sub> S*O <sub>4</sub>		
						Grain	Straw	Grain	Straw	
A. Sulphur Uptake Experiments										
Location										
20	1951	156	169	1.97	2.11	0.10	0.05	0.13	0.10	56
21	1951	116	123	1.43	1.53	0.12	0.05	0.11	0.12	53
18	1951	308	328	1.82	1.95	0.12	0.03	0.13	0.12	57
17	1951	83	103	2.24	2.08	0.11	0.04	0.11	0.10	58
16	1952	168	209	1.53	1.39	0.13		0.12		45
19	1952	157	198	1.89	1.93	0.13		0.14		41
22	1952	138	142	1.66	1.60	0.13		0.14		38
Total		1126	1272	12.55	12.59	0.84	0.17	0.88	0.44	348
Average		160.9	181.7 <sup>2</sup>	1.79	1.80	0.12	0.04	0.13	0.11	50
B. Feeding Depth Experiments <sup>3</sup>										
Location										
16	1952	145	187	—	—	0.10	—	0.12	—	35
19	1952	152	157	—	—	0.13	—	0.12	—	27
22	1952	138	142	—	—	0.13	—	0.15	—	8

<sup>1</sup>  $\text{Na}_2\text{S}^*\text{O}_4$  at 100 lb. per acre.<sup>2</sup> Statistically significant yield increase of  $\text{Na}_2\text{S}^*\text{O}_4$  fertilized over check.<sup>3</sup> The  $\text{Na}_2\text{S}^*\text{O}_4$  supplying 20 lb. sulphur per acre was placed at 10-inch depth.

TABLE 8.—UPTAKE OF FERTILIZER SULPHUR BY PLANTS ADJACENT TO AREAS TREATED WITH  $\text{Na}_2\text{S}_2\text{O}_4$ 

Location	Crop	Origin of sample	S per cent from fertilizer	
			1st cut	2nd cut
11	Alfalfa	6 in. from plot receiving 50 lb./ac. $\text{S}^*$	11.0	17
		12 in. from plot receiving 50 lb./ac. $\text{S}^*$	0.9	7
		24 in. from plot receiving 50 lb./ac. $\text{S}^*$	0.2	2
20	Wheat	12 in. from plot receiving 20 lb./ac. $\text{S}^*$	0.4	—
		24 in. from plot receiving 20 lb./ac. $\text{S}^*$	0.0	—
18	Oats	12 in. from plot receiving 20 lb./ac. $\text{S}^*$	1.2	—
		24 in. from plot receiving 20 lb./ac. $\text{S}^*$	0.0	—
17	Wheat	12 in. from plot receiving 20 lb./ac. $\text{S}^*$	1.3	—
		24 in. from plot receiving 20 lb./ac. $\text{S}^*$	0.2	—



### *Yield Experiments with Sulphur Containing Fertilizers*

Data for these experiments are summarized in Tables 9 and 10. Table 9 shows trends rather similar to those of Table 6 although the yield increases are not as great as many previously reported in Alberta (11). However, with the exception of flowers of sulphur, yield increases reported in Table 9 average close to 100 per cent for fields responding to fertilization. Such increases are highly profitable. The relatively poorer results from flowers of sulphur are probably due to the slowness of its transformation to sulphate. On legumes, no definite difference between the other fertilizers tested is established. This is not the case with grains. Table 10 shows a yield response to the ammonium phosphate 16-20-0 with a suggestion of response to ammonium sulphate. The results are in accord with fertilizer recommendations on these soils (4).

On two fields responding to sulphur fertilization of legumes (locations 11 and 20) fall and spring applications of the fertilizer treatments listed were compared. There was considerable fluctuation in yields but on the average there was no difference in yield increases.

### *Feeding-Depth Experiments*

A number of conclusions may be drawn from Table 11. When  $\text{Na}_2\text{S}^*\text{O}_4$  was placed at 10- or 22-inch depths, there was an appreciable uptake of fertilizer sulphur by alfalfa. When fertilizer was placed at both 10- and 22-inch depths there was uptake from both depths although the uptake from the 10-inch depth was the greater. Increasing depth of placement of the  $\text{Na}_2\text{S}^*\text{O}_4$  tended to result in a lower uptake of fertilizer sulphur in the first hay cutting but this was not evident in the second hay cutting. The technique employed here is similar to that described by Lawton *et al.* (7) and enables studying the rate and extent of root penetration and development as well as the uptake of nutrients. Uptake of  $\text{S}^{35}$  placed at various depths was much greater than that of  $\text{P}^{32}$  as reported by Lawton *et al.* The  $\text{S}^{35}$  content of second-cut hay was about equal for surface 10- and 22-inch depths in contrast to the sharply decreased uptake of  $\text{P}^{32}$  with increased depth of placement.

## GENERAL DISCUSSION

A comparison of the data reported with the sulphur content of similar plant materials as listed by Morrison (10) shows important differences. With the exceptions of plants grown on the Edmonton Plots and location 22, the amounts of this element are lower than those which Morrison lists. Straws showed the greatest difference, Morrison's figures being about fourfold those reported here.

The sulphur content of plant materials, especially of grain straws, may afford a means, other than field testing with fertilizers, of determining need for sulphur fertilization. However, considerable time would be required to establish whether such a method would be suitable or reliable. In the experiments reported here, soil analysis, proximity to legume fields responding to fertilization, and low sulphur content of grain straws, all

TABLE 9.—HAY AND SEED YIELD, NITROGEN AND SULPHUR CONTENT OF LEGUMES GROWN ON FARM FIELDS WITH VARIOUS SULPHUR CONTAINING FERTILIZERS IN 1951 AND 1952

Treatment <sup>1</sup>	Yield, ton/acre		N per cent		S per cent		Seed, lb. per acre
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	
<i>Locations not definitely responsive to sulphur fertilization (No. 5, 7, 8, 9, 14, and 15. Figure 1)</i>							
Check	1.2 (6) <sup>2</sup>	0.8 (3)	2.99 (5)	2.11 (2)	0.17 (5)	0.14 (2)	18 (1)
S	1.3 (6)	0.9 (3)	2.99 (5)	2.38 (2)	0.20 (5)	0.18 (2)	17 (1)
CaSO <sub>4</sub>	1.2 (6)	1.0 (3)	3.04 (5)	2.22 (2)	0.27 (5)	0.16 (2)	20 (1)
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.3 (6)	1.0 (3)	3.04 (5)	2.48 (2)	0.26 (5)	0.19 (2)	26 (1)
Na <sub>2</sub> SO <sub>4</sub>	1.3 (6)	0.9 (3)	3.05 (5)	2.49 (2)	0.27 (5)	0.17 (2)	21 (1)
16-20-0	1.5 (6)	1.0 (3)	2.97 (5)	2.25 (2)	0.27 (5)	0.16 (2)	21 (1)
Na <sub>2</sub> SO <sub>4</sub> + trace	1.2 (6)	0.9 (3)	3.05 (5)	2.33 (2)	0.25 (5)	0.17 (2)	20 (1)
<i>Locations definitely responsive to sulphur fertilization (No. 3, 4, 6, 10, 11, 12, and 13. Figure 1)</i>							
Check	0.8 (7)	0.9 (4)	2.44 (6)	2.76 (1)	0.10 (6)	0.12 (1)	62 (3)
S	1.0 (7)	1.0 (4)	2.65 (6)	2.93 (1)	0.13 (6)	0.13 (1)	60 (3)
CaSO <sub>4</sub>	1.4 (7)	1.8 (4)	2.91 (6)	3.12 (1)	0.21 (6)	0.14 (1)	109 (3)
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.4 (7)	1.7 (4)	2.84 (6)	3.30 (1)	0.20 (6)	0.17 (1)	99 (3)
Na <sub>2</sub> SO <sub>4</sub>	1.4 (7)	1.8 (4)	2.82 (6)	3.32 (1)	0.19 (6)	0.22 (1)	116 (3)
16-20-0	1.5 (7)	1.9 (4)	3.01 (6)	3.06 (1)	0.23 (6)	0.17 (1)	117 (3)
Na <sub>2</sub> SO <sub>4</sub> + trace	1.4 (7)	1.7 (4)	2.77 (6)	3.10 (1)	0.20 (6)	0.16 (1)	88 (3)

<sup>1</sup> With the exception of check all treatments were at rates supplying 20 lb. of sulphur per acre.<sup>2</sup> Data are averages for the number of locations indicated by the figures in parentheses.

TABLE 10.—YIELD, NITROGEN AND SULPHUR CONTENT OF GRAIN ON FARM FIELDS FERTILIZED WITH VARIOUS SULPHUR CONTAINING FERTILIZERS

Treatment	1952 data for grains (locations 16, 19, and 22)				Av. yield bu./ac. <sup>1</sup>
	Number of farms	Av. yield bu./ac.	N per cent (av.)	S per cent (av.)	
Check	3	39.5	1.63	0.13	40.0
Sulphur	3	43.2	1.63	0.16	41.9
CaSO <sub>4</sub>	3	43.7	1.70	0.15	41.2
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	3	49.4	1.61	0.13	44.1
Na <sub>2</sub> SO <sub>4</sub>	3	46.4	1.73	0.13	42.6
16-20-0	3	51.9	1.53	0.13	47.3 <sup>2</sup>
Na <sub>2</sub> SO <sub>4</sub> + trace	3	43.6	1.62	0.13	41.5

<sup>1</sup> Data for 7 locations 1951 and 1952.<sup>2</sup> Yield increase significant at 5 per cent point.

suggest that at least some of the grain fields tested would show this deficiency if cropped to legumes. However, grains in Alberta seldom show yield response to sulphur fertilization and this would greatly handicap efforts to develop a method of determining the need for such fertilization based on the sulphur content of grain straws. Since sulphur fertilization is highly profitable on responsive fields (4) it is very important to learn what farms do give yield increases when so fertilized.

The data reported afford a means of estimating the amounts of sulphur removed by crops on Alberta grey soils. Fertilized legume crops in the tests reported removed on the average about 10-14 lb. of sulphur per acre where two cuts of hay were taken. When both grain and straw are taken from the field removal by average farm grain crops will be about 4-7 lb. of sulphur. Where crop rotations employing both grains and legumes are followed on grey soils deficient in this element an average application of 10 lb. of sulphur per acre per year should generally meet crop needs. At Breton applications considerably less than this have proven highly satisfactory (11).

Application of gypsum, trace elements, and phosphorus in ammonium phosphate 16-20-0 did not result in yield increases above those attributable to sulphur in these materials. These results confirm previous tests in Alberta which have not revealed responses to lime or trace elements (5). Phosphorus fertilization of forage has frequently, but not consistently, resulted in increased yields of forage crops. Absence of any significant yield increase from phosphorus in the 13 farm tests on forage reported here is unexplained.

No evidence of radiation injury due to S<sup>35</sup> was found or observed. This does not rule out the possibility of such injury. However, the results of Penner (12) working with P<sup>32</sup> suggest that there is little probability of any consequential damage from S<sup>35</sup> radiation in these experiments since the amounts of S<sup>35</sup> used were comparatively very small.

TABLE 11.—SULPHUR CONTENT AND PERCENTAGE OF FERTILIZER SULPHUR IN ALFALFA FROM FIELDS  
HAVING VARIOUS PLACEMENTS OF  $\text{Na}_2\text{S}^*\text{O}_4$  AND  $\text{Na}_2\text{SO}_4$ , 1952

Location	Treatment	S per cent		Fertilizer S per cent	
		1st cut	2nd cut	1st cut	2nd cut
13	S* on surface	0.24	0.14	56	45
	S* at 10 in.	0.20	0.13	44	54
	S* at 22 in.	0.20	0.15	29	45
	S at 10 in. + S* at 22 in.	0.20	0.16	10	16
	S* at 10 in. + S at 22 in.	0.23	0.19	29	29
14	S* on surface	0.26	0.23	35	25
	S* at 10 in.	0.25	0.20	29	25
	S* at 22 in.	0.21	0.17	28	27
	S at 10 in. + S* at 22 in.	0.25	0.20	25	13
	S* at 10 in. + S at 22 in.	0.26	0.21	32	19

## NOTES:

- (a) S\* on surface, at 10 in. and 22 in. refers to  $\text{Na}_2\text{S}^*\text{O}_4$  supplying 20 lb. sulphur per acre placed at depths indicated.  
 (b) S refers to  $\text{Na}_2\text{SO}_4$  supplying 20 lb. sulphur per acre placed as indicated.



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# THE BORON REQUIREMENTS OF STONE FRUIT TREES<sup>1</sup>

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## ABSTRACT

A study was made of the syndromes associated with boron deficiency and excess on peach, apricot, prune, and cherry trees grown in sand culture. On the four types of fruit trees examined boron deficiency was characterized by a spring die back of branches. Symptoms of toxicity varied considerably: on peach, brown necrotic marks on the underside of the main rib of the leaf were most characteristic; on apricot, swelling of the nodes; on prune, cracking and sloughing off of bark on 2-year-old twigs; and on cherry, die back of terminals accompanied by gumming. Results of chemical analyses indicated that there was a close relationship between the boron content and expression of tree symptoms.

Since the discovery of the need for boron by apple trees in the fruit growing areas in the interior of British Columbia by McLarty (6) additional reports (5, 7, 9) have been made on the requirements of other types of fruit for this element. These papers describe symptoms associated with low available amounts of this nutrient and only one (7) details the symptoms when an excess of it is present. It is the purpose of this paper to review the symptoms associated with boron deficiency on peach, apricot, prune and cherry; to review those of boron toxicity on peach; and to describe the symptoms caused by an excess of this element on apricot, prune and cherry trees when grown in sand culture. Chemical determinations for boron were made on samples of tissues collected from trees receiving varying amounts of boron and some results are presented.

## EXPERIMENTAL

Peach (var. J. H. Hale), apricot (var. Wenatchee Morepark), prune (var. Early Italian), and cherry (var. Bing) trees were planted in screened and washed Okanagan lakeshore sand in each of 12 pans set in the laboratory grounds. One tree of each species was planted in each pan. The pans, which were circular, 5 feet in diameter, and 14 inches high, had a drain-hole in the bottom. They were made of galvanized iron and were coated with an asphalt paint. During the growing season the trees in the pans were irrigated with 48 litres of Hoagland's four salt plus micronutrients solution. There were six treatments, each duplicated, and these differed only in the amount of boron added to each. The treatments were as follows:

<i>Treatment 1</i>	No added boron
<i>Treatment 2</i>	0.01 p.p.m. boron
<i>Treatment 3</i>	0.5 p.p.m. boron
<i>Treatment 4</i>	1.0 p.p.m. boron
<i>Treatment 5</i>	5.0 p.p.m. boron
<i>Treatment 6</i>	10.0 p.p.m. boron

Tap water was used in the nutrient solutions and during warm weather additional water was added as required in order to prevent wilting. To

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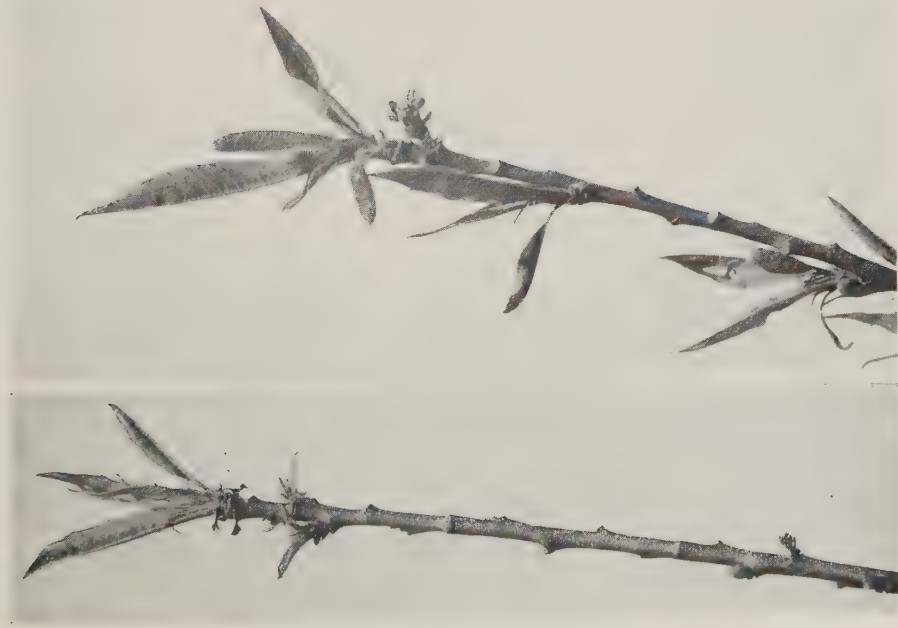


FIGURE 1. The response of a boron deficient peach tree to a spray containing this element. (*Left*) An unsprayed twig. (*Right*) A twig 10 days after spraying



FIGURE 2. Boron toxicity on apricot twigs. Note the greatly enlarged nodes. Some of the axial shoots are stunted and dead.



FIGURE 3. Stunted spur growth on prune due to an excess of boron. At the end of the growing season some of the leaves were only about one inch long.



FIGURE 4. Two-year-old prune twigs on which the bark cracked, curled outwards, and in some instances sloughed off. This is a characteristic symptom of boron toxicity on prune. The symptoms increase in severity from left to right.



lessen frost damage during winter months, the trees were protected by a 6-inch layer of shavings placed over the sand in the pans. This layer was removed each spring and the sand was flushed with tap water. For analyses, leaf samples from the median portion of the current season's growth were collected during the first week of October and twig samples after the leaves had fallen.

Observations of the symptoms of boron deficiency and toxicity were made at frequent intervals during the experiment which was carried on for 5 years. Boron determinations were made by either the curcumin method (8) or by a recent modification (2) of this procedure.

## OBSERVATIONS AND RESULTS

### *Peach*

Symptoms associated with boron deficiency and toxicity on peach trees growing under field conditions in the Okanagan Valley have been described (7). Both in the field and in sand culture the most typical symptoms of boron deficiency were a spring die back and a sloughing off of buds. In the latter they developed only on trees in the no added boron cultures and then not until the final year of the experiment. That year, during the first week of May these symptoms were extremely severe and it was believed that the trees would die. To prevent this from occurring one tree and portions of the other were sprayed with Polybor (sodium pentaborate) at the rate of 1 pound in 100 gallons of water. Four days after the treatment new tissue development was clearly discernible and growth continued for the remainder of the season although no further boron was added (Figure 1). The untreated portions died.

Boron toxicity was characterized by the appearance of small necrotic spots on the under side of the main rib of affected leaves and small cankers on 1- and 2-year-old twigs. On trees in the 10 p.p.m. cultures these symptoms appeared in the first year and were associated with a yellowing and premature dropping of the leaves. In the final year of the experiment a few leaves on the peach trees in the cultures receiving 1.0 p.p.m. boron exhibited typical symptoms of toxicity. The symptoms were first noted on the apical leaves. Other species of trees in these cultures were normal. The peach trees in the cultures receiving 10 p.p.m. boron died after 2 years and those in the 5 p.p.m. cultures after 4 years. No other trees died in these high boron cultures even though they exhibited marked boron toxicity symptoms. The dying of terminal shoots and adjacent leaves was a symptom of boron toxicity on all the trees tested.

The boron content of leaves and twigs varied with the amount added to the sand cultures (Table 1, parts (a) and (b)). The amount present in leaves from trees in the no added boron culture varied from 6 to 12 p.p.m. on an air dry weight basis and these values compare favourably with those reported (7) for leaves from trees growing in the field. Peach leaves from trees growing in the 10 p.p.m. culture contained 200 p.p.m. boron. This value is somewhat higher than that reported (7) for affected leaves from trees in orchards. The latter value was 168 p.p.m. Under the condition of the experiment, peach and cherry trees had the ability to absorb boron more readily or at least in greater amounts than did apricot and prune. How-

TABLE 1.—THE BORON CONTENT OF TISSUES FROM STONE FRUIT TREES GROWN IN SAND CULTURE

Treatment	Boron in nutrient solution p.p.m.	Year	Boron content, p.p.m. dry weight basis			
			Peach	Apricot	Prune	Cherry
(a) Leaves (average of duplicates for three successive years)						
1	—		10	—	—	19
2	0.01		18	27	31	55
3	0.5		55	36	38	75
4	1.0		46	34	35	80
5	5.0		121	64	54	141
6	10.0		200*	81	61	167
(b) Twigs (average of duplicates for one year)						
1	—		16	—	—	17
2	0.01		26	14	14	28
3	0.5		32	25	28	45
4	1.0		63	62	64	76
5	5.0		—	63	82	136
6	10.0		—	90	85	197
(c) Leaves (average of duplicates)						
2	0.01	1951	42	27	46	66
2	0.01	1952	28	18	27	55
2	0.01	1953	28	—	19	42

\* Average of duplicates for one year only.

ever, with peach so much was absorbed that the trees in the high boron cultures died. In part (c) of Table 1 the values for the boron content of leaves collected for three successive years from trees growing in the 0.01 p.p.m. cultures show a steady decrease. From the regularity of this decrease it is believed that the amount of boron present within the tree tissues was being steadily used up. This indicates that 0.01 p.p.m. boron in cultures would be inadequate for continued growth of stone fruit trees.

### *Apricot*

Symptoms associated with boron deficiency in apricot have been described (1, 5). The winter following the planting of the trees in the sand cultures was very severe and temperatures of  $-22^{\circ}$  F. were recorded. At this temperature the apricot and prune trees in the no added boron sand cultures died, possibly because their resistance to cold in the absence of sufficient of this nutrient was decreased. Those receiving 0.01 p.p.m. survived. Typical symptoms of boron deficiency on apricot leaves and twigs did not occur but on fruit on a tree in a 0.01 p.p.m. culture they did appear and were characterized by longitudinal cracking of the epidermal layers.



FIGURE 5. Die back on cherry due to a boron deficiency. The terminal bud commenced to grow in the spring but later, following leaf defoliation, died.

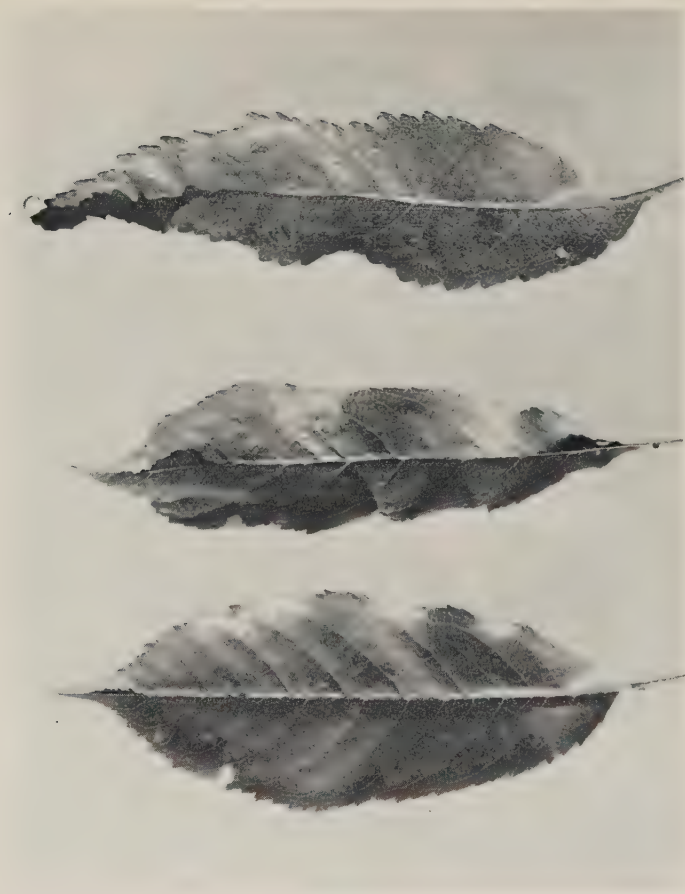


FIGURE 6. Leaves from cherry trees growing in a low boron sand culture. Affected leaves are narrow and the serrations are irregular. The leaf on the left is normal.



FIGURE 7. Die back of cherry twigs caused by an excessive amount of boron. Gumming is a common symptom.



FIGURE 8. Cherry leaves showing symptoms of boron toxicity. Note the necrotic areas, sometimes near the midvein and at others in the body of the leaf.



The most striking feature associated with boron toxicity in apricot was the greatly enlarged nodes on 1- and 2-year-old twigs (Figure 2). This enlargement was associated with a shortening of the internodes and usually with gumming. On 2-year-old and occasionally on 1-year-old twigs the bark became severely cracked and sloughed off. The tips of many twigs died during the summer and the leaves near the tips turned black and dropped. On small twigs, on leaf petioles, and on the lower side of the main vein of the leaf there was a cankering and necrosis of the epidermal layers. Axial shoots frequently commenced to grow from the swollen nodes, but they were stunted and sometimes died within a few weeks (Figure 2). In orchards, swelling of nodes was not always observed even when other toxicity symptoms were present.

In the 5- and 10-p.p.m. sand cultures several blossoms opened but only a few fruits set. The fruit was normal in size, shape, and colour but ripened prematurely. On the surface of the fruit there were a few small, 1-3 millimetre diameter, irregularly shaped scab-like protuberances, which sloughed off as the fruit ripened. A similar condition was found on fruit in various orchards and it was shown that it was associated with a boron content greater than 95 p.p.m. on a dry weight basis. In orchards and in sand cultures this condition was frequently associated with a dying back of terminals and a blackening of apical leaves.

The results of some analyses for boron are presented in Table 1. The sample of leaves from trees in the 10 p.p.m. culture showed toxicity symptoms and contained 94 p.p.m. boron.

### *Prune*

The prune trees in the no added boron sand cultures died during the first winter when temperatures of  $-22^{\circ}$  F. were recorded. There were no symptoms of deficiency prior to their death nor did any appear in the tissues of trees growing in the 0.01 p.p.m. culture. In the field, symptoms of boron deficiency are similar to those found on other fruit trees (7).

Symptoms of boron toxicity occurred on the prune trees growing in the cultures receiving 5 and 10 p.p.m. boron. Swelling of the nodes was prominent but not as marked as that found on apricot. In the spring, a bud or buds at or near a terminal broke and grew normally until about mid-June when the tips died and a small amount of die back occurred. This condition did not change during the remainder of the growing season. On 2-year-old twigs, spurs developed at some of the swollen nodes but at others growth was late in starting, slow in developing, and at the end of the season leaves on these spurs were frequently only about an inch in length (Figure 3). The bark on 2-year-old twigs and more occasionally on 1-year-old twigs cracked, the edges of the bark near the crack curled upwards, and peeled off (Figure 4). No gumming occurred on prune trees.

Leaves on trees affected with boron toxicity felt coarse to the touch. The mid rib was greatly thickened and the leaf tissues adjacent to it were of a bronze colour. Occasionally, small cankers appeared along the back of the midvein. These lesions were light brown in colour as contrasted to those on peach which were dark and pronounced. Small, irregularly-shaped portions of the leaf, positioned near and with their major axis perpendicular to the midvein, became necrotic and fell out. Affected and

adjacent leaves were small and irregularly developed. The leaf margins on many leaves were rolled upwards and especially when near the terminal of a branch became necrotic and blackened.

The fruit on prune trees showing boron toxicity symptoms ripened much earlier than did that on healthy trees. It was normal in shape but was small in size. When boron toxicity was apparent blossoming was delayed. The results of some analyses are presented in Table 1.

### *Cherry*

Cherry trees growing in sand cultures containing no added boron showed deficiency symptoms on twigs and leaves in the fourth and fifth year of the experiment. Terminal growth was only 0 to 8 inches, whereas that on trees containing adequate amounts of boron was 16 to 24 inches. Typical die back symptoms, in which some buds failed to develop and others opened, then shrivelled and died, were present. In some instances the terminal commenced to grow and the twig elongated for a few weeks, then following defoliation, died (Figure 5). Leaves on affected limbs were narrow and the serrations were irregular (Figure 6). Blossoms did not develop even though fruit buds were sometimes present.

Dying back of twigs was a symptom of boron toxicity and on cherry was always associated with gumming (Figure 7). Gumming also occurred along some of the main branches and on the trunk when the severity of the toxicity was marked. Nodes were normal. Leaves from trees growing in high boron cultures were normal in size and shape but there were small necrotic areas in the tissues lying along the main vein and occasionally in the body of the leaf (Figure 8). The opening of blossom buds was greatly retarded and no fruit set. In the final year of the experiment growth on the trees in the 10 p.p.m. cultures was negligible and boron toxicity symptoms were marked. The results of some chemical analyses are summarized in Table 1.

### ACKNOWLEDGEMENTS

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# AN ANOMALOUS CROSS BETWEEN *HORDEUM LEPORINUM* AND *HORDEUM VULGARE*<sup>1</sup>

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## ABSTRACT

All evidence leads to the conclusion that there was some kind of a union as a result of pollinating *H. leporinum* Link ( $2n = 28$ ) by *H. vulgare* L. ( $2n = 14$ ). Three  $F_1$  plants which failed to head in the field were grown as clones in a greenhouse and produced two main kinds of plants *viz.*, those with  $2n = 28$  which resembled the female parent in most characters but without leaf pubescence; and those with  $2n = 14$  which resembled the male parent in many characters but which had a brittle rachis like the female parent and also a winter growth habit and seed dormancy. There were other plants including dwarfs, one plant with abnormal nodes, one with abnormal leaf growth and a few which died. Progeny of the vulgare type segregates were not homozygous for growth rate, spike shape and awn roughness. They are resistant to powdery mildew and possibly to other leaf diseases. The vulgare types can be crossed with *H. vulgare* and chromosome pairing is normal. A suitable explanation cannot be given for the mechanism involved in this union nor has it been possible, as yet, to repeat the cross. The new barley types produced may be of great value to plant breeders.

## INTRODUCTION

This paper is a special report from a project on interspecific hybridization in *Hordeum*. It concerns two species only, *H. leporinum* Link and *H. vulgare* L., and hence no literature review pertaining to hybridization between other species will be given. *H. leporinum* Link is a sub-species of *H. murinum* L., and the two are very similar. A few attempts have been made to cross *H. murinum* and *H. vulgare* and in some cases a few shrivelled seeds were obtained but all were inviable (2).

The results in this report are special because there has been a transfer of genetically-controlled characters, as usually occurs in a cross, but there is no direct evidence to prove that a cross has occurred. The material will be designated as hybrid material but the actual behaviour after pollination is not clearly understood. However, after careful consideration, it was deemed advisable to report this occurrence, both because of its unusual nature, and because some of the plants obtained may be quite valuable as breeding stocks. Then, too, some reader may have encountered a somewhat similar behaviour and may be able to supply a satisfactory solution to the problem.

## MATERIAL

The North American material classified as *H. murinum* is mostly *H. leporinum* (1). The species shows great variability mainly in the expression of quantitative characters. The 28 chromosomes of *H. leporinum* regularly form 14 pairs at meiosis and the spikes are completely fertile.

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Therefore, it does not behave as an autotetraploid. The particular species used in this study has been carried in the genetic stocks of the Cereal Crops Division for several years as "*H. murinum* Odessa". Its origin is unknown. The plants were studied recently by W. G. Dore, Division of Botany and Plant Pathology, Science Service, Canada Department of Agriculture, and identified as *H. leporinum* Link. The species is classified as an annual but at Ottawa it does not head during the summer if planted in the spring. Some plants have survived the winter at Ottawa and headed the following summer. Leaves and stems are pubescent, the spike has a brittle rachis and the awns are rough. In the seeds the aleurone is blue. The plants are resistant to mildew and the leaf diseases normally present in the field and in the greenhouse.

The *H. vulgare* spikes used for pollination were taken from hybrid populations comprising the following crosses: (Bolivia  $\times$  Chevron)  $\times$  Montcalm<sup>2</sup>; (Bolivia  $\times$  Chevron)  $\times$  Fort<sup>2</sup>; (Anoidium  $\times$  (Bolivia  $\times$  Chevron)  $\times$  Montcalm; and Anoidium  $\times$  Montcalm<sup>2</sup>. All hybrids were glabrous, some had rough awns and others were smooth or semi-smooth. They all had a normal barley spike, that is, with a non-brittle rachis. Some had a blue aleurone and some white. Resistance to mildew and leaf diseases varied from one hybrid to another but none exhibited the complete resistance of the *Hordeum leporinum* type. None of the hybrids nor their parents carry any known genes for winter growth habit.

#### PROCEDURE

Approximately 70 emasculated spikes of *H. leporinum* were bagged with spikes of *H. vulgare* that were shedding pollen during the greenhouse season of 1951-52. Since previous attempts to cross the two species indicated that it was most difficult or impossible to do, no record was kept of the hybrid designation of each specific male parent. Stimulation of the embryos on *H. leporinum* occurred frequently but the small "seed-like" growths withered and died. Ten spikes were treated in a somewhat different manner. The spikes were wrapped in absorbent cotton immediately after pollination and kept moist for about 10 days. The liquid used to moisten the cotton was drawn from the top portion of a pail of water to which had been added a handful of 2-12-6 fertilizer. Seed development was better in the wrapped spikes and 86 plump seeds were obtained. No explanation is offered for either the procedure or the result.

The 86 seeds showed no signs of deterioration after 10-12 days. Hence no attempt was made to excise the embryos for embryo culture. The seeds were stored in a refrigerator at approximately 40° F. for 8 days and then placed on blotting paper in Petri dishes to germinate at room temperature.

#### DESCRIPTION OF ORIGINAL "HYBRIDS"

Three seeds germinated after one month but grew slowly. The remaining seeds did not germinate. Eventually the three small plants were transplanted to pots and later placed in the field in May. They grew to small grassy clumps about 5 inches in height by fall. Their grassy appearance indicated that they might have arisen by self-fertilization.





FIGURE 1. A pot of the original clone I showing leporinum and vulgare types. The latter are indicated by arrows.

FIGURE 2. Mature spikes of leporinum types from clone I.

FIGURE 3. Mature spikes of vulgare types from clone I.



However, the plants were glabrous and different from the leporinum parent which was pubescent. The three clumps were taken into a greenhouse in the fall of 1952, labelled as clones I, II, and III, and broken up so that each tiller was planted separately.

They grew slowly but eventually stems began to form and elongate. None of the plants headed until late February, 11 months after seed germination. The plants varied greatly in growth morphology as indicated below.

*Clone I:*

There were two types of plants:

(1) Most of the plants resembled *H. leporinum* except that they were taller and more vigorous. The plants were glabrous and each spike had a brittle rachis (Figure 2). The chromosome number was  $2n = 28$ .

(2) It was surprising to find four vulgare type plants with vigorous growth, wide pale green leaves, and coarse culms (Figure 1). The spikes had a brittle rachis like *H. leporinum*, rough awns, and grains with a white aleurone (Figure 3). The chromosome number was  $2n = 14$ .

*Clones II and III:*

A range of plant types was produced. There were a few short non-vigorous plants which had broad leaves and the general appearance of *H. vulgare*, but they failed to head and died. The others resembled *H. leporinum* in spike type but they varied in size from dwarf types to others the size of *H. leporinum*. Each spike had a brittle rachis. The plants were glabrous. The chromosome number was  $2n = 28$ .

One plant in clone III produced only a mass of leaves some of which measured 23 inches in length. The plant did not head. Another plant with leporinum type spikes had a loop in the stem directly above the upper node.

All plants from clones I, II, and III, including the four vulgare types, were resistant to powdery mildew caused by *Erysiphe graminis*. The plants were grown in a house used continuously for the creation of mildew epidemics and none showed any signs of infection.

#### PROPAGATION OF VULGARE TYPE SEGREGATES

One of the four vulgare type spikes produced in clone I was destroyed accidentally. Seed from the other three was planted in the field in the spring of 1953, one month after harvest. Germination was slow and uneven. Out of 129 seeds planted, 97 germinated and 76 plants survived. They grew very slowly and reached a height of 6-8 inches by fall. The plants were not decumbent in growth habit, nor did they grow upright like spring barley. They tillered quite profusely compared to normal barley. In the fall of 1953, they were moved to a greenhouse and placed in pots. Growth was very slow and some plants had a pale yellowish-green colour as noted in the original plants. Several had shortened culm internodes which made the leaves bunch together. Fifty-nine of the 76 survived. The heading period extended throughout the month of March which was 10-11 months after seeding. There was considerable variation in spike shape and

plant height. The spikes were classified into eight types as illustrated in Figure 4. The number of plants falling in classes 1-8, respectively, was: 3, 6, 10, 7, 9, 20, 3 and 1. Thus, seven of them had a brittle rachis, i.e., type 4. The awns on all spikes were classified as rough, but there were variations in degree of roughness.

Chromosome preparations were made from root tips of the growing plants. The sizes and shapes of the chromosomes were identical with those of standard barley varieties. There were no irregularities in pairing behaviour in the spikes examined at microsporogenesis. Seed setting was regular.

An attempt was made to germinate the seeds from the 59 plants four weeks after maturity but they were in a dormant condition and did not germinate. Samples were placed in a refrigerator at 40° F. for one week but still they failed to germinate. After another week in the refrigerator all seeds germinated. Half of the seed from each of the 59 spikes was then planted in the field in the spring of 1954. Each plant produced a large number of light green decumbent leaves similar to those of winter barley when seeded in the spring (Figure 5). Additional seed of each of the 59 spikes was seeded in early September near the spring seeded material and both lots will have a test for winterhardiness.

The plants showed no disease symptoms during the entire summer, although powdery mildew, leaf spotting diseases, stem and leaf rust were abundant on check varieties nearby. Some leaf rust appeared on the plants in late fall.

#### BACKCROSSING THE "HYBRIDS" TO *H. VULGARE*

Attempts to backcross the plants ( $2n = 28$ ) from clones I, II, and III, which resemble *H. leporinum*, to *H. vulgare* have failed so far. However, there is always plenty of embryo stimulation.

The plants ( $2n = 14$ ) from clone I which resembled *H. vulgare* produced good pollen which gave perfect seed set when used to pollinate *H. vulgare*. One anther was removed from each floret to make the following four crosses:

Hybrid 55 = ( (Bolivia × Chevron) × Fort<sup>2</sup> ) × Clone I

Hybrid 56 = Montcalm × Clone I

Hybrid 57 = ( (Newal × Peatland) × O.A.C.21 ) ×  
(Anoidium × Rabat) ) × Clone I

Hybrid 58 = (Valentine × Fort<sup>2</sup>) × Clone I .

The  $F_1$  plants were grown during the summer of 1953. The seeds germinated over a 2-3 week period. The plants were very vigorous and were free from rust, powdery mildew, and leaf spotting diseases which were present on check varieties in adjacent rows. Several spikes were examined cytologically. There were no pairing irregularities at first metaphase of meiosis. Other stages were also normal. There was normal seed setting.

The  $F_1$  plants were examined for the brittle rachis character, awn roughness and aleurone colour. Numbers were not large enough to establish ratios because this study was not progressing according to plan but as unexpected events and circumstances dictated. Moreover, the brittle rachis character is difficult to determine except when the spikes are





FIGURE 4. Eight spike types produced by propagating the three original vulgare type spikes; types are numbered 1-8 from left to right.



FIGURE 5. Portion of interspecific hybrid nursery 1954; winter growth habit of vulgate type segregates illustrated in foreground;  $F_2$  populations of *H. vulgate*  $\times$  vulgate type segregates (Hybrids 55-58) in background.



TABLE 1.—AWN AND ALEURONE CHARACTERISTICS OF  $F_1$  PLANTS OF *H. vulgare*  $\times$  VULGARE TYPE SEGREGATES

	Awns		Aleurone	
	Rough	Smooth	Blue	White
Hybrid 55	23	6	29	—
Hybrid 56	17	14	31	—
Hybrid 57	8	1	9	—
Hybrid 58	15	4	19	—

extremely brittle. The determination of the degree of brittleness may be affected by stage of maturity and whether or not the plants are grown in the greenhouse or field. Both brittle and non-brittle spikes were easy to find but many could not be classified with accuracy. The data on awn roughness and aleurone colour are given in Table 1.

The female parents had a blue aleurone and the male parents had a white aleurone. It should be noted that the segregation for aleurone colour refers to general spike colour. That there were some white seeds on these spikes was shown by the segregation in the  $F_2$  population. Inheritance studies have shown that there is frequently a single dominant gene for blue aleurone. Thus the mode of inheritance for aleurone colour is normal. Inheritance studies have shown also that the rough awned character is usually dominant to the smooth awned character. In these crosses the male parents were rough awned and the female parents semi-smooth or smooth. The preponderance of rough awns indicates a normal mode of inheritance for this character. The appearance of some smooth awned types indicates that the male parent was not homozygous. This is another indication, along with observations on growth rate and spike type, that the vulgare segregates are not homozygous.

The  $F_2$  populations of hybrids 55-58 were grown during the summer of 1954. Growth was vigorous and the plants were remarkably free of diseases. This was a most outstanding demonstration because it was a year when powdery mildew, leaf spotting diseases, and the rusts were severe. A few families did have a moderate infection of powdery mildew and leaf rust. Each population segregated for spike type, for awn roughness, for the brittle rachis character, and for spring, intermediate and winter habit of growth. Details on the inheritance of the characters which the vulgare type segregates possess, and their reaction to diseases will be published separately.

#### ***H. VULGARE* $\times$ PROGENY OF THE ORIGINAL VULGARE TYPE SEGREGATES**

Twenty-seven of the 59 plants were used as male parents to pollinate a number of spring barley hybrids which were available in March 1954. The  $F_1$ 's were grown during the summer of 1954. Since the crosses were made on smooth awned hybrid lines, some of which were in advanced generations but not necessarily homozygous, ratios cannot be relied upon.

The 27 groups of  $F_1$  plants were by no means similar. Individually the groups were classified as being: all spring types; a mixture of winter and intermediate types; a mixture of spring and late intermediate types; a mixture of spring and winter types; mostly winter types; late spring types; or all winter types. The plants segregated for the brittle rachis character. It is especially significant that one plant was used to pollinate four closely related hybrid lines of spring barley and from one cross the  $F_1$  plants were mostly winter types with some intermediates; from two other crosses they were mostly spring types with some winter types; and from the fourth cross they were all spring types. Such results were common in this material.

### DISCUSSION

It is apparent that when *H. leporinum* was crossed with *H. vulgare* an unusual type of union occurred as exemplified by the following observations:

1. The vulgare type segregates had a winter habit of growth.
2. The spikes of the vulgare type segregates had a brittle rachis.
3. The seed produced on the vulgare segregates was extremely dormant.
4. The vulgare segregates were not homozygous as shown by the segregation resulting from crosses using these types (see previous sections).
5. The vulgare segregates have 14 chromosomes which are identical with those of other barley varieties, *i.e.*, they are indistinguishable morphologically and they pair readily and regularly with the chromosomes of *H. vulgare*.
6. The leporinum type segregates have 28 chromosomes that readily form 14 closed bivalents at meiosis as is found in *H. leporinum*.
7. The leporinum type segregates have glabrous leaves both when grown in the greenhouse and in the field.
8. All plants, both vulgare segregates and the leporinum types which arose from this cross (union), have resistance to powdery mildew and to some other leaf diseases.
9. In addition to the vulgare segregates, other peculiar plants were obtained, notably dwarfs, long leafed plants and those with abnormal nodes.

When no sterile spikes with 21 chromosomes were produced on any of the plants, it was temporarily decided that no cross had been effected and that some mistake had occurred whereby *H. vulgare* plants were growing in the pots along with selfed *H. leporinum* plants. Later, when the winter habit, brittle rachis and disease resistance were so apparent in the vulgare segregates, and when leporinum types were now glabrous, it was necessary to reverse the decision because there was no other possible source for the material. Possibly the segregates with 21 chromosomes were those types which died before producing spikes.

In 1948 Huskins (4) startled the cytological world with an article entitled "Segregation and reduction in somatic tissues". Since then, further reports have stressed chromosome segregation into groups (reductional grouping) rather than crossing over between chromosomes in root tips. Briefly the theory is that after treatment by drugs, and antibiotics, or by occurring naturally in a small proportion of cases, the chromosomes separate into groups at mitosis and then form aneuploid cells out of these groups. Some of the aneuploid cells remain viable and by further divisions can form a plant or part of a plant with an altered make-up of chromosomes. Somewhat similar behaviour has been reported in other cereals by workers in other countries (3, 5). In addition, some unpublished investigations are known to the present authors.



It is likely that if any such "reductional grouping" could have occurred in this material, then the cells with the haploid complement from the original parents would be the most viable and competitive ones *i.e.*, those with 14 chromosomes from *H. leporinum* and those with 7 from *H. vulgare*. In accordance with the original theory these cells, when doubled, would produce plant tissue cells with 28 and 14 chromosomes. However, it is the haploid complement which is doubled and therefore the plants would be entirely homozygous; they were not. Unfortunately then, this theory is not entirely satisfactory.

Chromosome breakage, occurring in the original hybrid group of chromosomes, could lead to reciprocal interchanges between the chromosomes of the two species. If the exchange of the chromosome parts occurred after chromosome doubling, the plants could be heterozygous; if it preceded doubling, the plants would be homozygous. There was no reduction in pairing (as examined at metaphase of meiosis) in either the original plants or in crosses involving the chromosomes of the vulgare types and the chromosomes of other barleys. The only basis for argument, then, is if the sectors were small enough so as not to interfere in the pairing relationships of the chromosomes. The many suppositions required to establish and uphold this theory have forced its relegation to a place of minor importance.

The inheritance of rough awns and aleurone colour seems to be controlled in a simple Mendelian fashion. This does not contradict previous reports. The brittle rachis character was presumably transferred from *H. leporinum* to the vulgare type segregates and from there was transferred to spring and intermediate barley types by crossing to ordinary barley. The winter habit of growth also arose in some anomalous fashion and since then has continued to segregate in an equally enigmatic manner. Possibly these characters are controlled by cytoplasmic genes, but there is no substantiating evidence in other reports. The argument in favour of cytoplasmic inheritance or of the interaction of one set of chromosomes in an abnormal cytoplasm can be justified by emphasizing that this "cross" is a novel one and such an unusual combination may have many possibilities.

Naturally, attempts have been made to repeat the cross. Seeds have been obtained by pollinating the original collection of *H. leporinum* formerly known as *H. murinum* Odessa. No plants have been produced from the seeds. Other collections of *H. leporinum* have not set seeds when pollinated. It is doubtful if such a behaviour will occur again and yet, one is stimulated by the fact that all 3 seeds, out of 86, which did germinate, behaved in a similar fashion. There is further enthusiasm because of the valuable plant breeding material that has arisen from this union. It may have new genes for winterhardiness, which are certainly needed in barley, and it may have new genes for disease resistance.

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# INTERACTION OF SODIUM AND POTASSIUM ON GROWTH AND MINERAL CONTENT OF FLUE-CURED TOBACCO<sup>1</sup>

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## ABSTRACT

An experiment with flue-cured tobacco grown in sand culture showed that sodium-potassium combinations in the nutrient supply had significant effects on yield and mineral content of the plants. The effects of variations in the K supply on yield was highly significant; that of Na was just below the level of significance. The Na  $\times$  K interaction effect on yield was highly significant: at zero-K supply, yield increased with increased Na supply; but, at high-K supply, yield decreased with increased Na supply. When K was deficient, symptoms of K deficiency on the plants were reduced in severity by the addition of Na to the nutrient supply. It was indicated that, when K was deficient, Na partially supplemented or replaced K in the nutrition of the plants.

Na and K accumulated in the plants in direct relation to the supply. K depressed the uptake of Na by the plant and the two elements had an additive effect in depressing the uptake of Ca, Mg and P.

## INTRODUCTION

The exact role of sodium in plant nutrition is not well understood. Although sodium is not generally considered to be an essential nutrient element for plants, many crops absorb this element in large quantities and show significant growth responses to it. The effect of sodium on plants has been interpreted variously. Sodium and potassium are in the same group of chemical elements and some investigators have noted interrelations between the response of plants to these two elements in the fertilizer (1, 2). Plant responses to sodium have been noted when the supply of potassium is considered adequate (4) but are more pronounced when potassium is deficient (2, 3).

The influence of sodium on plant growth varies with the plant species and with other cations present in the root medium. Whether this element performs, in some plants, a specific function or merely assists in the function of potassium in the metabolic process of the plant is unknown. There is the additional possibility that sodium may influence plant growth through its activity in the soil complex.

Little is known regarding the influence of sodium on the growth and quality of tobacco. The object of this investigation was to determine the effects of sodium in the nutrient medium, when supplied in combination with potassium and without potassium, on growth and composition of the flue-cured type of tobacco.

## MATERIALS AND METHODS

White Mammoth, a variety of flue-cured tobacco, was used in this experiment. The seedlings were grown in sand in 3-inch pots for nine

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TABLE 1.—YIELD DATA IN GRAMS OF PLANTS GROWN ON VARYING PROPORTIONS OF POTASSIUM AND SODIUM

Treatment No.	Treatment p.p.m.		Average fresh weight per plant					Average dry weight per plant				
	K	Na	Top leaves	Bottom leaves	Total leaves	Stalk	Total	Top leaves	Bottom leaves	Total leaves	Stalk	Total
1	0	0	67	62	129	54	183	9.4	14.9	24.3	5.4	29.7
2	0	50	78	46	124	71	195	10.9	13.3	24.2	6.4	30.6
3	0	200	94	71	165	119	284	14.1	18.5	32.6	13.1	45.7
4	50	0	210	242	452	293	745	42.0	46.0	88.0	44.0	132.0
5	50	50	239	261	455	280	735	47.8	41.0	88.8	42.0	130.8
6	50	200	215	196	411	269	680	49.5	39.2	88.7	40.4	129.1
7	200	0	267	259	526	353	879	58.7	46.1	104.8	63.5	168.3
8	200	50	251	270	521	353	874	57.7	48.6	106.3	60.0	166.3
9	200	200	230	257	487	314	801	48.3	43.7	92.0	56.5	148.5
L.S.D. P = 0.05			13	12	24	16	42					

weeks. During that time, they were supplied with a complete nutrient solution, previously developed (5), in which all the nutrient elements were within the optimum range of concentration for the best growth of tobacco. It did not contain sodium. This solution was compounded from the following reagent-grade salts: calcium nitrate, potassium dihydrogen phosphate and magnesium sulphate. It contained Ca at a concentration of 240 p.p.m.; Mg, 45 p.p.m.; K, 195 p.p.m.; N, 168 p.p.m.; S, 60 p.p.m., and P, 155 p.p.m. The minor elements B, Mn, Cu, and Zn were each added to the solution at the rate of 0.5 p.p.m. and Fe was added at the rate of 3 p.p.m.

The seedlings were transplanted to 3-gal. glazed, self-draining jars, each containing 40 lb. of ground Nepean sandstone. One plant was grown in each jar and was supplied with one litre of nutrient solution per day, applied by the constant flow, drip method.

The nine nutrient solutions used in the experiment comprised three levels of Na (0, 50, and 200 p.p.m.) in factorial combination with three levels of K (0, 50, and 200 p.p.m.). The other elements were supplied at an optimum concentration for the best growth of tobacco as determined previously (5) as follows, in p.p.m. of the solution: Ca, 240; Mg, 45; P, 155; N, 168; S, 90; B, 0.5; Zn, 0.5; Mn, 0.5; Cu, 0.5; and Fe, 3. Six replications of each treatment were used in randomized arrangement and the plants were grown under greenhouse conditions.

The plants were harvested two weeks after topping. Fresh weights were recorded for three fractions of the tissue: the top leaves, the bottom leaves, and the stalk. These fractions were cut into small sections and thoroughly mixed. Samples were weighed out for dry-weight determinations and for chemical analysis and dried in an oven at 70° C. The methods used in the chemical analysis were the official methods of the A.O.A.C. (6).

## RESULTS AND DISCUSSION

### *Growth of Plants*

The fresh-weight and dry-weight yield data are presented in Table 1. An analysis of variance was performed on the fresh-weight data and the least significant difference between any two means at the 5 per cent level is shown in the table.

The Na supply over the range studied was not a significant source of variation in fresh-weight yield. Variations in K supply had a highly significant effect on yield: at each Na level, the yield increased progressively with successive increments of K. The Na  $\times$  K interaction was highly significant.

Table 2 presents the fresh-weight yield data of the total leaves for the nine treatments and the mean yields at each level of Na and K. The least significant difference between the yield means for main effects of Na and K was determined to be 15.4 grams. A comparison among the means shows that increasing K caused significant yield increases but the effect of Na was not significant. This table shows the pattern of the significant Na  $\times$  K interaction: at the zero-K level, the yield increased from 129 gm. to 165 gm. with increased Na; but, at the 200-p.p.m.-K level, the yield decreased from 526 gm. to 487 gm. with increased Na.



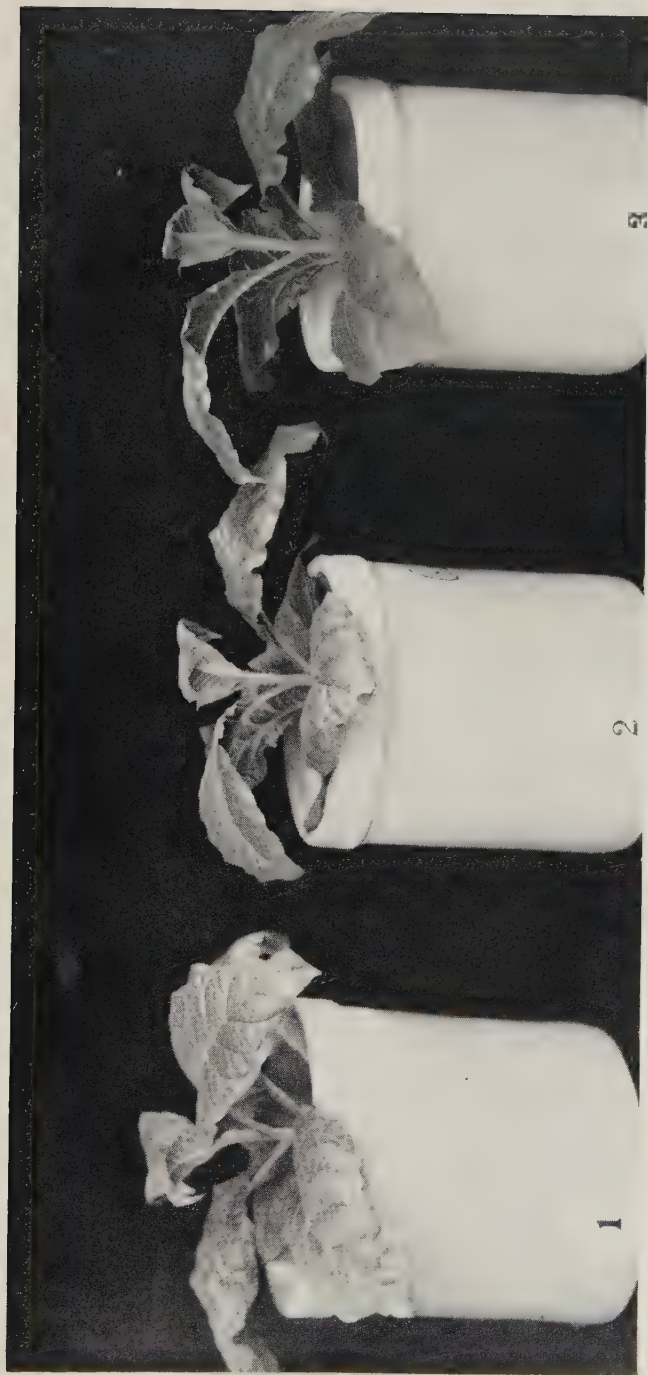


FIGURE 1. During the early growth stage of tobacco plants which received no potassium, added sodium apparently replaced potassium: Treatment 1, O K-0 Na, severe potassium-deficiency symptoms. *Middle:* Treatment 2, O K-50 p.p.m. Na, mild potassium-deficiency symptoms. *Right:* Treatment 3, O K-200 p.p.m. Na, no potassium-deficiency symptoms. In later growth, all plants manifested potassium-deficiency symptoms.



FIGURE 2. On tobacco plants which received 50 p.p.m. potassium in the nutrient supply, the addition of sodium reduced potassium-deficiency symptoms. *Left:* Treatment 4, 50 p.p.m. K-O Na, mild potassium-deficiency symptoms. *Middle:* Treatment 5, 50 p.p.m. K-50 p.p.m. Na, very mild potassium-deficiency symptoms. *Right:* Treatment 6, 50 p.p.m. K-200 p.p.m. Na, no potassium-deficiency symptoms at time photograph taken, but mild deficiency symptoms developed in later growth.

TABLE 2.—MEAN FRESH-WEIGHT YIELDS OF LEAVES OF TOBACCO FOR 9 Na-K NUTRIENT COMBINATIONS AND YIELD MEANS AT EACH FACTOR LEVEL, SHOWING MAIN EFFECTS OF Na AND K

	0 p.p.m. K	50 p.p.m. K	200 p.p.m. K	Yield means for main effect of Na
	gm.	gm.	gm.	gm.
0 p.p.m. Na	129	453	526	369.3
50 p.p.m. Na	125	455	521	367.0
200 p.p.m. Na	165	411	487	354.3
Yield means for main effect of K	139.7	439.7	511.3	—

L.S.D. ( $P = 0.05$ ) for Na and K means = 15.4 gm.

The dry-weight data indicate a growth response to Na and K similar to that shown by the fresh-weight data. The yield increased appreciably with increasing K supply at all levels of Na supply in all fractions of the tissue with the exception of the top leaves when K was increased from 50 to 200 p.p.m. at high-Na supply (treatments 6 and 9). The dry-weight data present further evidence of the Na  $\times$  K interaction. When the Na supply was increased from zero to 200 p.p.m., the yield for all fractions of the tissue increased at the zero-K level, decreased at the high-K level, but remained relatively unaffected at the intermediate-K level.

Characteristic symptoms of potassium deficiency developed on the plants which received no potassium (treatments 1, 2, and 3). However, the deficiency symptoms developed first and were most severe in treatment 1 which received neither sodium nor potassium (Figure 1). By contrast, the deficiency symptoms appeared later in the growth period and were less severe in treatments 2 and 3 which received 50 and 200 p.p.m. sodium, respectively. Also, the plants which received 50 p.p.m. potassium (treatments 4, 5, and 6) manifested symptoms of potassium deficiency, though less severely than in the zero-potassium treatments. Here, also, the expression of potassium-deficiency symptoms was delayed and reduced in severity by the addition to the nutrient solution of 50 and 200 p.p.m. sodium, respectively, in treatments 5 and 6 (Figure 2). Potassium-deficiency symptoms were not observed in treatments 7, 8, and 9 in which the supply of potassium was considered to be adequate for growth (5). Thus, it is indicated that sodium partially supplemented or replaced potassium in the nutrition of the tobacco plants when potassium was deficient.

#### MINERAL CONTENT OF PLANT TISSUE

The results of the analyses of the leaves and stalks for Na, P, K, Ca, Mg, and S are presented in Table 3. The data representing the concentration of the elements are expressed as percentage of oven-dry weight of the

TABLE 3.—EFFECTS OF VARYING PROPORTIONS OF SODIUM AND POTASSIUM IN THE NUTRIENT SUPPLY ON MINERAL CONTENT OF TOBACCO

Treatment No.	Treatment		Analysis expressed as percentage oven-dry weight														
	K p.p.m.	Na p.p.m.	Top leaves					Bottom leaves					Stalk				
			Na	K	Mg	Ca	P	S	Na	K	Mg	Ca	P	S	Na	K	Mg
1	0	0	0.09	0.62	1.18	5.67	0.74	0.54	0.09	0.49	1.26	6.50	0.90	0.50	0.10	0.61	0.64
2	0	50	0.11	0.57	1.13	5.45	0.77	0.51	0.12	0.46	1.28	6.31	0.88	0.52	0.63	0.49	0.60
3	0	200	0.24	0.55	0.93	4.48	0.75	0.43	0.51	0.42	1.18	5.59	0.79	0.49	1.20	0.50	0.54
4	50	0	0.06	1.01	0.61	3.93	0.75	0.47	0.11	0.50	0.91	5.40	0.72	0.34	0.06	1.18	0.59
5	50	50	0.15	1.06	0.67	3.83	0.65	0.53	0.17	0.48	1.02	5.61	0.72	0.38	0.22	1.10	0.51
6	50	200	0.36	1.08	0.57	3.29	0.60	0.44	0.47	0.51	0.89	4.68	0.64	0.36	0.54	1.10	0.52
7	200	0	0.09	2.64	0.40	2.19	0.59	0.50	0.08	2.83	0.63	3.42	0.61	0.48	0.06	2.21	0.56
8	200	50	0.10	2.66	0.34	2.02	0.55	0.45	0.12	2.44	0.55	3.13	0.53	0.48	0.08	2.30	0.52
9	200	200	0.15	3.17	0.27	1.77	0.42	0.48	0.18	2.71	0.41	2.75	0.45	0.50	0.20	2.39	0.46



tissues. Variations in the concentration of each of Na and K in the nutrient solutions had a significant effect on the concentration of mineral elements in the plant tissues.

At all rates of K supply, each successive increase in the supply of Na resulted in an increase in the content of Na in the top leaves, bottom leaves and stalk. The greatest correlation between Na supply and Na content of the plant tissue was found in the stalk. In general, each increment of Na depressed the content of each of Mg, Ca, and P in all three fractions of the tissue. In the high-K treatments, plants receiving 200 p.p.m. Na had a higher K content in the top leaves and stalks than did those receiving no Na. Otherwise, Na had little influence on the uptake of K. There was no definite relationship between Na supply and S content.

At all rates of Na supply, each increment of K resulted in an increase in the K content of all three fractions of the tissue. Conversely, high-K supply depressed the accumulation of Na, Ca, Mg and P in all fractions of the tissue and S in the stalk tissue. At high-Na supply, increasing K depressed the Na content of both bottom leaves and stalks, but the data for the top leaves were somewhat irregular.

Na and K had an additive effect in depressing uptake of Ca, Mg and P. The content of these three elements reached the lowest level in both leaves and stalks in treatment 9 with combined high-Na and high-K supply.

The results of this experiment indicate that the flue-cured tobacco tested gives only a slight response to Na in the nutrient supply. The alleviation of K-deficiency symptoms on the plants by added Na suggests that there is a supplementary relation between K and Na in the nutrient supply with regard to the growth of the plants. However, only a partial substitution of Na for K in the nutrition of the plant was indicated and no beneficial response of tobacco to the inclusion of Na in the nutrient supply can be expected at adequate levels of K.

It is evident that, at deficiency levels of K, the presence of Na in the nutrient supply may suppress the expression of K-deficiency symptoms on the plants and, consequently, interfere with the interpretation of experimental results. It is, therefore, necessary to exclude Na from the nutrient supply when investigating the K nutrition of tobacco.

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# SOME FACTORS AFFECTING EARLINESS IN THE TOMATO

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## ABSTRACT

Pollination and maturity dates were recorded for a large number of tomato fruits grown under glass in the spring for the production of  $F_1$  hybrid seed. The earliness of 46 varieties as expressed by number of days (1) from seed planting to first bloom, and (2) from pollination to ripening, was determined. The variety Farthest North was exceptionally early in both categories.

Xenia does not play an important role in earliness of tomato fruits.

Counts of the seeds in the individual fruits showed: (1) no relationship between seed content of the fruits and earliness of the varieties; (2) that, within a variety, the fruits with the most seeds matured fastest—on the average an increase of 50 to 70 seeds hastened maturity by one day; (3) that the first three trusses had fewer seeds per fruit than later trusses.

Position of the truss, in itself, does not affect rate of ripening. However, fruits at the distal end of the truss take longer to mature than those at the proximal end.

## INTRODUCTION

Earliness is of prime importance to tomato growers in Ontario. In the spring greenhouse crop and the outdoor crop for early market, the first picking may be worth twice as much per pound as the second. Growers of contract acreage want early varieties so that they may spread the labour of harvest and make certain of harvesting the full crop before frost. Processors desire a large, high quality, early variety so that they may spread the period of heavy deliveries and at the same time increase the quality of their pack. Colour is one of the main factors determining quality in tomatoes and, as the fruits usually are a deeper red in August and early September, early maturity is very important to the processors.

Earliness is influenced by four main periods in the development of the plant: (1) seed germination to blossom; (2) blossom to fruit setting; (3) fruit setting to ripening, and (4) first harvest to peak harvest. Varieties studied by workers in Michigan (7) showed little hereditary difference in either period 1 or period 3. Most of the difference in earliness between early, mid-season and late varieties was in the interval between blossoming and fruit setting. Early varieties were early in those studies because they set fruit at lower temperatures than late varieties. The period between first harvest and peak harvest is shorter in determinate varieties than in indeterminate varieties, as the fruit clusters are separated by fewer leaves.

## MATERIALS AND METHODS

For the production of  $F_1$  seed, 34, 16 and 15 tomato varieties were grown in the greenhouse in the springs of 1947, 1948, and 1949, respectively. Seed was planted about December 15 and the plants transplanted to ground beds about February 1. Each individual flower was emasculated one or

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two days before it would normally open. The petals were left on, and when they were fully expanded the flower was pollinated, and tagged with a record of the cross and date of pollination. In 1948 the plant number was also recorded, and in 1949 the truss number, and position of the flower on the truss. Usually only the first five flowers on each truss were pollinated. Nearly all pollinations were made between 8 and 11 a.m. All fruits were picked at corresponding maturities. The change in colour from greenish yellow to orange takes place rapidly in the tomato. The fruits were considered mature at the time of this colour change and picked every morning. The picking date was recorded on the tag. The seed from each fruit was removed, making sure that none was missed. Excess moisture was removed with the aid of a kitchen strainer. The seed, one seed thick, was then spread on mimeograph paper to dry. As the gelatinous matrix about the seeds dried, it fastened them securely to the paper. This facilitated both counting and storage.

## RESULTS

### *Number of Days to First Bloom*

The period from germination to first bloom varied from year to year but certain varieties generally were earlier than others. A summary of the data from 1947 to 1949 is given in Table 1. The very early varieties, such as Farthest North and many of the other determinate varieties, bloom early. It will also be noticed that, in the early spring in the greenhouse, most varieties fall in the "medium time" category. In this group, some of the early varieties, such as Valiant, Harkness and Red Jacket, take about the same length of time as some of the late varieties, such as Indiana Baltimore and Garden State. Certain varieties, e.g. Connecticut No. 3, are late, because the first flower cluster fails to develop under the low light conditions of early spring.

TABLE 1.—EARLINESS OF TOMATO VARIETIES AS EXPRESSED BY AVERAGE TIME FROM SEED PLANTING TO FIRST BLOOM

Bloom date	Season	Varieties
Before March 7	Very early	Farthest North, Earlinorth, L-3700-II, Redskin.
March 8-15	Early	All Red, Best of All, Bounty, Firesteel, Mingold, Pearl Harbor.
March 16-23	Medium	Camdown, Clark's Early, "Cooper's Special", Dick Locke, Early Baltimore, Garden State, Grothen's Globe, Harkness, Indiana Baltimore, Geneva John Baer, Longred, Marglobe, Michigan State Forcing, N.D. Special, Ontario, Diener's Pepper, Ponderosa, Pritchard, Que. No. 8, Que. No. 9, Que. No. 13, Red Jacket, Riverside Favorite, Scarlet Dawn, Splendid, Sioux, Stokesdale, Stokesdale No. 4, Valiant.
March 24-31	Late	Cardinal, Comet, Conn. No. 3, Golden Jubilee, Oxheart, Rutgers, Wisconsin 55.

### *Number of Days from Pollination to Ripening*

The rate of ripening depends in part on the weather. The average number of days from pollination to maturity was determined by weekly intervals beginning March 8 (Table 2). Farthest North, Earlinorth, and Redskin were omitted from the calculations as they mature much more rapidly than the other varieties. In 1947, flowers pollinated the middle of March required 71 days to mature whereas those pollinated the end of April required only 60 days. In 1948, pollinations made March 17-23 took 63.2 days and those made April 7-13 took 58.8 days. In 1949, fruits pollinated March 10-16 took about 7 days longer to ripen than when pollinated April 29-May 4. This difference in time may be attributed to the greater light duration and intensity or to higher temperatures as the season progressed.

The number of days from pollination to maturity was adjusted for season and year, using March 24 to 30, 1948, as a base. The maturation times of those pollinated March 31-April 6, 1948, for example, were brought to the base by adding two days, and those pollinated March 17-23, 1947, by subtracting nine days. After this adjustment, the earliness of the tomato varieties as expressed by days from pollination to ripening is as shown in Table 3. The data were based on about 50 to 60 fruits from each

TABLE 2.—INFLUENCE OF POLLINATION DATE ON NUMBER OF DAYS  
FROM POLLINATION TO RIPENING

Year	Pollination date									
	Mar. 10-16	Mar. 17-23	Mar. 24-30	Mar.-Apr. 31 6	Apr. 7-13	Apr. 14-20	Apr. 21-27	Apr.-May 28 4	May 5-11	May 12-18
1947	71.9	70.4	67.4	65.5	63.0	61.7	59.8	59.9	—	—
1948	62.4	63.2	61.6	59.2	58.8	—	—	—	—	—
1949	62.5	59.5	57.3	58.1	57.6	55.4	53.7	55.5	48.4	48.3

TABLE 3.—EARLINESS OF TOMATOES AS EXPRESSED BY DAYS  
FROM POLLINATION TO RIPENING

Number of days	Varieties
50-55	Farthest North.
56-60	Earlinorth, Redskin.
61-65	Best of All, Camdown, Clark's Early, Conn. No. 3, Dick Locke, Early Baltimore, Early Rutgers No. 78, Grothen's Globe, Harkness, Indiana Baltimore, Geneva John Baer, L-3700-II, Marglobe, Michigan State Forcing, Mingold, N.D. Special, Oxheart, Diener's Pepper, Ponderosa, Pritchard, Que. No. 8, Que. No. 9, Red Jacket, Sioux, Splendid, Stokesdale, Wisconsin 55.
66-70	All Red, Bounty, Cardinal, "Cooper's Special", Firesteel, Garden State, Golden Jubilee, Longred, Ontario, Pearl Harbor, Que. No. 13, River-side Favorite, Rutgers, Scarlet Dawn, Stokesdale No. 4, Valiant.



variety. Farthest North, a small-fruited variety, was outstanding in its rate of ripening. Its flesh turned colour 12 to 15 days quicker than that of most varieties. Certain early market varieties, such as Bounty and Firesteel, were among those which took longest to mature.

### *Influence of Pollen Parent on Rate of Ripening*

In 1947, dates from pollination to maturity were all rearranged according to the pollen parents and compared as to weekly pollination dates. There was no apparent difference between varieties. The data for Farthest North in 1948 were also analysed (Table 4). There is a trend in the data to suggest that pollen of Farthest North may have hastened maturity very slightly, and also that fruits from selfed flowers may have ripened more slowly than fruits from hybridized flowers. However, the data are not

TABLE 4.—RATE OF FRUIT MATURITY IN 1948 OF RECIPROCAL CROSSES INVOLVING FARTHEST NORTH, COMPARED WITH DATA FOR OTHER VARIETIES

	Pollination date							
	Mar. 3-9	Mar. 10-16	Mar. 17-23	Mar. 24-30	Mar.-Apr. 31 6	Apr. 7-13	Apr. 14-20	Apr. 21-27

#### Farthest North selfed

Number of days	54.5	50.5	—	—	46.5	—	—	—
Number of fruits	2	2	—	—	4	—	—	—

#### Farthest North as seed parent

Number of days	51.2	48.8	46.5	47.2	47.6	46.7	—	—
Number of fruits	13	8	6	9	18	4	—	—

#### Farthest North as pollen parent

Number of days	67.0	—	61.9	60.5	62.2	55.8	59.0	64.5
Number of fruits	1	—	11	11	5	10	3	3

#### Other varieties as both seed and pollen parents

Number of days	64.2	62.4	63.2	61.6	59.2	58.8	56.1	59.3
Number of fruits	6	73	160	174	151	126	52	28

#### Other varieties selfed

Number of days	—	63.7	63.6	62.2	57.9	57.6	57.0	52.0
Number of fruits	—	6	16	20	15	17	11	1

extensive enough to show significant differences. Even if significant differences could be shown, there would be no practical application of the knowledge since the tomato is naturally self-pollinated. Xenia does not play an important role in earliness in the tomato.

#### *Influence of Number of Seeds on Rate of Maturation*

The various varieties were compared with respect to the average seed count and the average number of days to maturity. The 1949 data arranged in order of seed content are given in Table 5. Some of the very early varieties, such as Farthest North and Redskin (1948 data), had exceptionally low seed counts. There was also a trend for the later varieties to have higher seed counts. This may be because the late canning types have larger fruits than the early market types. The regression of days on seed count was not significant. In a breeding program, the selection of lines with a given maturity, e.g. early, midseason or late, cannot be made on the basis of the seed content.

TABLE 5.—VARIETIES COMPARED AS TO NUMBER OF SEEDS AND NUMBER OF DAYS TO MATURITY IN 1949

Variety	Average number of seeds	Average number of days	Variety	Average number of seeds	Average number of days
Garden State	352	59.2	Ontario	200	57.8
Wisconsin 55	297	52.1	Marglobe	195	52.3
Firesteel	292	60.6	Conn. No. 3	195	55.6
Cardinal	279	59.5	Pritchard	190	54.9
Scarlet Dawn	262	58.6	Rutgers	180	58.1
Stokesdale No. 4	228	56.1	Stokesdale	172	56.3
Ponderosa	215	53.6	Early Rutgers No. 78	167	56.0
Diener's Pepper	210	55.8			

l.s.d. 5 per cent for number of seeds—82.4

l.s.d. 5 per cent for number of days— 5.0

Regression = 0.17 days per 10 seeds (not significant).

It was noted in 1947 that many of the fruits which took longest to mature contained only a few seeds and were usually rather small. The data for Camdown in 1948, showing the relationship between days to ripen (adjusted for season) and number of seeds, are given in Table 6. In general, the greater the seed content of the fruits the quicker the fruits matured.

TABLE 6.—COMPARISON OF THE DAYS TO RIPEN AND SEED CONTENT OF CAMDOWN FRUITS IN 1948

	Days to ripen						
	56-58	59-61	62-64	65-67	68-70	71-73	74-76
Number of fruits	11	15	18	9	3	1	1
Average number of seeds	327	278	242	263	158	88	204

TABLE 7.—INFLUENCE OF NUMBER OF SEEDS ON DAYS TO MATURITY IN 1948 AND 1949

Variety	Number of fruits	Mean number of seeds	Mean number of days	Regression days/10 seeds
Bounty	57	293	63.9	-0.16**
Camdown	60	262	61.7	-0.17**
Cardinal*	46	279	59.5	-0.26**
Conn. No. 3	55	261	56.2	-0.17**
Conn. No. 3*	42	195	55.6	-0.13**
Early Baltimore	64	214	59.5	-0.00
Early Rutgers No. 78*	57	167	56.0	-0.05
Farthest North	68	86	49.0	-0.36**
Firesteel*	71	292	60.6	-0.33**
Garden State*	57	352	59.2	-0.22**
Harkness	67	262	59.5	-0.41**
Longred	55	257	62.7	-0.17**
L-3700-II	59	235	60.7	-0.12**
Marglobe*	72	195	52.3	-0.35**
Ontario*	57	200	57.8	-0.06
Pepper (Diener's)*	59	210	55.8	-0.01
Ponderosa*	93	215	53.6	-0.19**
Pritchard	63	225	61.8	-0.16**
Pritchard*	111	190	54.9	-0.22**
Red Jacket	55	268	60.7	-0.31**
Redskin	58	155	56.3	+0.10
Rutgers	60	221	60.9	-0.27**
Rutgers*	101	180	58.1	-0.31**
Scarlet Dawn	81	262	58.6	-0.08
Sioux	65	268	59.5	-0.18**
Stokesdale	55	244	58.4	-0.19**
Stokesdale*	40	172	56.3	-0.06
Stokesdale No. 4*	99	228	56.1	-0.16**
Valiant	45	267	62.2	+0.00
Wisconsin 55	64	295	60.0	-0.17
Wisconsin 55*	77	297	52.1	-0.06

\* 1949, others 1948.

\*\* Significant beyond the 1 per cent level.

In Table 7 the influence of the number of seeds on the rate of maturity of the fruit is given for 26 varieties. The relationship is expressed by the regression coefficient "b". This gives the number of days that the time to mature varies with every increase of 10 seeds. For example, for every increase of 10 seeds, fruits of the variety Connecticut No. 3 matured 0.17 days faster in 1948, and 0.13 days faster in 1949.

Twenty-two regressions were negative and very highly significant—much beyond the 1 per cent level. Seven were negative but not significantly so. The other two were positive but not significantly so. It would appear that some other factor—probably disease such as blossom-end rot or mosaic—was affecting these varieties.

The average effect of seed count on earliness was about 0.19 days per 10 seeds in 1948, and 0.15 days per 10 seeds in 1949. This means that, on the average, an increase of 60 seeds per fruit hastened maturity by one full day.

*Relation between Truss Position, Seed Count and Number of Days to Maturity*

The average number of seeds per fruit and the average number of days for the fruit to ripen were calculated for each truss in 1949. The data are given in Table 8. In 1949, the average seed content per fruit increased from the first to the fourth truss and then remained constant up to the seventh truss.

The number of days to maturity varied significantly with the position of the truss. In general, this variation coincided with the pollination dates. Hence, any increased rate of ripening in the upper trusses can be attributed to weather conditions and to increased seed count. Position of the truss, in itself, does not affect rate of ripening.

TABLE 8.—RELATION BETWEEN TRUSS POSITION, SEED COUNT AND NUMBER OF DAYS TO MATURITY IN 1949

	Truss position							l.s.d. 5 per cent
	1	2	3	4	5	6	7	
Number of fruits	205	206	193	155	133	95	75	—
Number of seeds per fruit	172	207	255	268	264	270	264	33.2
Number of days to maturity	59.1	57.2	56.1	55.1	57.2	51.7	50.6	2.7

Regression =  $-0.87^{**}$  days per 10 seeds

$^{**}$  Significant beyond the 1 per cent level.

TABLE 9.—RELATION BETWEEN SEED CONTENT, DAYS TO MATURITY AND POSITION OF THE FLOWER ON THE TRUSS FOR SOME VARIETIES GROWN IN 1949

	Flower position						l.s.d. 5 per cent	Regression, days/10 seeds
	1	2	3	4	5	6		

Number of seeds per fruit

Cardinal	450	242	298	159	142	279	116.8	
Conn. No. 3	211	125	236	185	273	196	107.2	
Marglobe	234	183	162	206	212	196	62.2	
All varieties	261	223	223	204	202	224	47.6	

Number of days to maturity

Cardinal	54.5	56.8	60.3	68.0	63.7	59.5	5.8	$-0.26^{**}$
Conn. No. 3	54.5	54.9	55.3	57.4	54.3	55.4	3.3	$-0.13^{**}$
Marglobe	49.1	51.5	52.6	56.0	59.0	52.6	5.9	$-0.35^{**}$
All varieties	54.1	55.2	57.3	60.7	59.3	57.2	2.8	$-1.02^{**}$

$^{**}$  Significant beyond the 1 per cent level.



*Relation between Position of the Flower on the Truss, Seed Content and Days to Maturity*

In picking greenhouse tomatoes it was observed that the first fruits on the top trusses would be ripening at the same time as the last fruits on the bottom trusses. This delay in maturity was not associated with a corresponding delay in time of blooming, and was not always associated with low seed count. In 1949, records were taken of the position of the fruit on the truss. Usually six flowers were pollinated on each truss. The proximal flower was designated Number One and the distal flower Number Six. The data for the varieties Cardinal, Connecticut No. 3 and Marglobe, as well as a summary for the varieties, are given in Table 9.

Since the six flowers in the trusses do not show a significant difference in the number of seeds and they do show a significant difference in the number of days to maturity, this indicates that some other factor is operating. The regression of days per 10 seeds accounts for some of the difference in days to maturity but not all of it. The difference is not associated with weather as weather acts in the opposite direction. It was shown (Table 2) that, the later in the spring that pollination takes place, the quicker the fruits mature. The delay in maturity of the distal fruits of the trusses may possibly be associated with degree of shading or, more likely, with the fruits being located towards the end of the nutrient supply. This theory is supported by the well-known phenomenon that frequently only five or six fruits develop on large inflorescences, even if many more flowers are pollinated.

Some varieties, e.g., Cardinal and Connecticut No. 3, had a significant decrease in the number of seeds in the second flower. In the spring of 1954, the flowers of the tomatoes growing in the greenhouse were closely examined. Frequently the second flower of the first truss was aborted or poorly developed. It is possible that this abnormality is carried by all trusses of some varieties to a greater or less degree.

## DISCUSSION

The greater the number of seeds in a fruit, the more rapidly will it develop. Quite aside from such considerations as larger fruit and greater uniformity of size and shape of the fruit, adequate pollination may make four or five days' difference in the time of ripening. This was confirmed in an experiment conducted in the spring of 1954 (2). The use of an electric vibrator to pollinate greenhouse tomatoes was compared with the commercial practice of shaking the plants. Not only were there greater weights of ripe fruit from the vibrated plants in the early harvests but the number of ripe fruits was also increased.

Evidently some hormone is being produced by the seeds which speeds up the ripening process. Many investigators (3, 5, 6, 7) have found that early yields of tomatoes are increased by applying growth regulators such as naphthoxyacetic acid and *p* chlorophenoxyacetic acid. There is usually very little increase, if any, in total yield. Growth regulators are most effective in increasing early yield during cool seasons when the night temperature is below 59° or 60° F. In other words, they shorten the period from blossom to fruit setting. No reports have been found where the effect of

growth regulators between pollination and maturity has been investigated. The closest study to this is a thesis by Cann (1). He treated the flower clusters with several growth regulators, including *p* chlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid, when 40 per cent of the flowers were open. A significant increase in number of ripe fruits at the first two pickings was obtained. He also gives the percentage of fruits which contained no seeds, some seeds, and many seeds. The whole increase in early yield was attributable to the seedless fruits, i.e. those which probably were derived from unpollinated flowers. Apparently the growth regulators tested did not increase the rate of ripening but merely initiated fruit development earlier. Marrè and Murneek (4) found that, for the first eight days after treatment, "application of the growth regulators *p*-chlorophenoxyacetic acid or the ethyl ester of indoleacetic acid to the cut surface of the style of emasculated flowers produced effects strikingly similar to those induced by pollination and fertilization including starch synthesis, a decrease of sucrose, and an increase of reducing sugars". Either the auxins produced by the seeds later in the development of the fruit act in a different manner than the growth regulators applied to the surface of the fruit, or the seeds continue to produce the auxins throughout development whereas the synthetic regulators are inactivated.

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# THE DISTRIBUTION AND PATHOGENICITY OF THE FUNGI ASSOCIATED WITH CROWN AND ROOT ROTTING OF ALFALFA IN MANITOBA<sup>1</sup>

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## ABSTRACT

The distribution of a number of fungi associated with rotting of alfalfa roots and crowns was determined with respect to the soil type, season, age of stand, and part of the root system from which they were isolated. The pathogenicity of the more prevalent fungi was studied on seedlings and mature plants. *Pythium debaryanum*, *Rhizoctonia solani*, and *Fusarium oxysporum* proved to be the most important seedling pathogens. Spring pathogens of mature plants included *Ascochyta imperfecta*, inciting a crown bud rot and root rot, *Plenodomus meliloti*, and *Cylindrocarpon ehrenbergi*. *R. solani*, inciting a crown bud rot, was the most important summer pathogen. *Pyrenochaeta terrestris* in sandy soils and *Fusarium* spp. in heavy black soils were prevalent in the summer, but are believed to be secondary invaders. *Stagonospora meliloti*, isolated predominantly from older stands, incited a crown rot and vascular discoloration.

## INTRODUCTION

Diseases affecting the crown and roots of alfalfa (*Medicago sativa* L.) have been of major concern to growers in Manitoba for a number of years. Stands of this crop are capable of surviving for 15 years or longer, but because of these diseases the majority of the fields show a progressive deterioration after the second year. By the end of the fourth or fifth year, stands are so unproductive that they can no longer be maintained with profit. As re-establishment is usually difficult, this decrease in productivity is particularly important in areas where alfalfa is grown for seed or in permanent pastures.

Numerous investigations on the cause of these crown and root rots have been made in various parts of the world, and many organisms have been held responsible. The literature on this subject has been reviewed recently by Erwin (12). In Manitoba, however, diseases of alfalfa received very little attention until about 1940. Previous to that year, the annual reports of the Canadian Plant Disease Survey listed scattered references to foliage diseases of this crop, but no mention was made of root or crown rotting pathogens. In 1940, Cherevick (6) completed a study of the fungi involved in crown and root rotting of sweet clover in Manitoba and Minnesota in which he reported that the major organism concerned in the disease was *Rhizoctonia solani* Kühn. In subsequent years, surveys, isolations, and inoculations on alfalfa led him to the conclusion that "a strain of *R. solani* is the main, if not the sole, causal agent of crown rot" (7). In view of Cormack's conclusion (10) that a complex of fungi is involved in the disease, it was thought advisable to make a more extensive study of the disease in Manitoba.

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The present investigation has followed the trend in research on root rots which has been towards a more complete knowledge of all the organisms associated with the injury. It was designed to determine the fungi associated with rotting of different parts of the root system of alfalfa plants of different ages, their distribution in different soil types, their relative prevalence at various times of the growing season, and their pathogenicity on alfalfa.

#### MATERIALS AND METHODS

The alfalfa fields selected for the survey were located in each of three important alfalfa-growing areas in Manitoba. These areas differ widely in soil type. The soil in the southeast corner of Manitoba varies from a gray wooded to a sandy type of soil which is low in organic matter. The Interlake region is composed of shallow soils with a high calcium content. These two areas are excellent for the production of alfalfa seed. The third area, in the Red River valley, contains soils which are black, heavy textured, and high in organic matter. Alfalfa is grown there mainly as a hay crop. The soils of all three areas are alkaline in reaction.

In each of these areas, one field each of first-, second- and third-year stands was selected. These were visited twice during the summer and once in the fall of 1951, and once in the spring and twice during the summer of 1952. On each occasion 100 plants were dug at random in each field and isolations were made from lesions appearing on the crown, primary roots, noncambial roots and from the lowest point of discoloration in the vascular region. In 1953, seedlings were grown in soil from each area and isolations made from damped-off seedlings and from lesions appearing on seedlings one month old. Sections of diseased tissue were surface sterilized in 2 per cent sodium hypochlorite and plated on potato sucrose agar. Diseased seedlings were placed in running tap water for 4 hours before plating. All plates were held at room temperature except those containing sections obtained in the spring which were incubated at 10° C. All fungus growths from these sections were transferred to slants for identification.

The pathogenicity of the seven most prevalent fungi isolated from seedlings was determined in the greenhouse by means of the jelly glass technique described by Halpin *et al.* (14), modified slightly. They mixed the agar plate cultures directly with the sand, but in the present experiments it was found that a more even distribution of the inoculum was achieved if the cultures were first macerated. Sixteen agar cultures, 4 each of 4 isolates of one species, were placed in a Waring Blendor along with sterile distilled water added at the rate of 25 ml. per plate and macerated for 3 minutes. This suspension was then mixed with sufficient sterile silica sand to half fill 16 tumblers.

Mature plant tests were conducted in controlled temperature tanks held at 16°, 20°, 24°, and 28° C. Ranger alfalfa plants, which had been grown from surface-sterilized seed in steam-sterilized soil for 14 weeks, were transplanted into half-gallon crocks containing a mixture of sterile soil and sand-cornmeal inoculum. Before being transplanted, the roots of these plants were also dipped into a suspension of mycelium and spores of the fungus. Ten plants per crock were planted in duplicate at each temperature. The plants were examined for disease lesions at the end of 6 weeks.



The pathogenicity of 10 of the most prevalent fungi isolated from mature plants during the survey was tested in this manner.

### EXPERIMENTAL RESULTS

#### *The Associated Fungi*

The predominant fungi encountered during the course of this survey are included in Table 1 and comprise approximately 70 per cent of the total isolates. The majority of the species have been reported previously on alfalfa from other regions and the remainder are usually regarded as common soil fungi.

*Pyrenochaeta terrestris* (Hansen) Gorenz, Walker & Larson, the incitant of pink root of onion, has not been previously recorded from alfalfa although it has been isolated frequently from sweet clover (*Melilotus indica* (L.) All.) roots in Louisiana (5).

Species of *Fusarium* are among the most commonly isolated fungi associated with diseased roots of alfalfa, but many of the reports appearing in the literature are of an observational nature only and the isolates are not identified to species. In the present survey over 30 per cent of the total

TABLE 1.—DISTRIBUTION IN RELATION TO SOIL TYPE OF THE FUNGI ISOLATED FROM ALFALFA ROOTS IN MANITOBA DURING THE SUMMER MONTHS OF 1951 AND 1952

Fungus	Percentage of total isolates <sup>1</sup>		
	Heavy black	Sandy	High lime
<i>Pyrenochaeta terrestris</i>	7	43	4
<i>Fusarium acuminatum</i>	14	15	15
<i>Ascochyta imperfecta</i>	6	8	5
<i>F. oxysporum</i> var. <i>redolens</i>	11	1	6
<i>F. oxysporum</i>	8	3	2
<i>Cephalosporium</i> sp.	4	Trace	8
<i>F. solani</i>	11	Trace	Trace
<i>Cylindrocarpon ehrenbergi</i>	3	3	5
<i>Stagonospora meliloti</i>	Trace	3	7
<i>Rhizoctonia solani</i>	3	1	3
<i>Plenodomus meliloti</i>	3	1	1
Miscellany identified	14	9	24
Miscellany unidentified	16	13	20
Total	100	100	100
Total number of isolates	986	988	394

<sup>1</sup> Averages for the isolates obtained from the crown, vascular region, primary roots, and noncambial roots of second- and third-year stands.

isolates were members of this genus, of which *F. acuminatum* Ell. & Ev. accounted for about one-half. This species is frequently isolated from diseased roots in Alberta (9, 16). *F. oxysporum* Schlecht. var. *redolens* (Wr.) Gordon has not been identified previously in isolates from North America, but has been reported on alfalfa in Europe (26). *F. oxysporum* has appeared infrequently in reported isolations from diseased alfalfa roots in North America. From alfalfa roots in Virginia, Fergus and Valleau (13) obtained several isolates which they placed collectively in the Section Elegans. *F. solani* (Mart.) App. & Wr. has been isolated from alfalfa in New Mexico (25), California (12), Virginia (13), and Europe (17). In Manitoba it has been isolated from sweet clover (*Melilotus alba* Desv.) roots (2). Other species of *Fusarium* isolated included *F. sambucinum* Fuckel f. 6 Wr., *F. equiseti* (Corda) Sacc., *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W. G. Sm.) Sacc., *F. poae* (Pk.) Wr., *F. meresmoides* Corda and *F. dimerum* Penz.

*Ascochyta imperfecta* Pk. has been found associated with diseased alfalfa roots in Alberta and Saskatchewan and has been isolated from soil under alfalfa in Alberta (11). It has not been reported previously on alfalfa roots in Manitoba but it is the most important incitant of foliage disease of this crop in the province.

Species of *Cephalosporium* have not been previously reported on this crop but are common soil inhabitants in Manitoba (1).

Four species of *Cylindrocarpon* were isolated of which *C. ehrenbergi* Wr. was the most prevalent. *C. radicicola* Wr., *C. obtusisporum* (Cke. & Hark.) Wr. and *C. olidum* Wr. were isolated much less frequently. The only previous record of these fungi on alfalfa roots is that of Cormack (8). He has isolated *C. ehrenbergi* from diseased roots in Alberta and Saskatchewan and from soil in Manitoba. The other three species have been reported from Alberta only, and have not been isolated from soil in Manitoba.

*Stagonospora meliloti* (Lasch) Petr., although widely distributed as a leaf-spotting pathogen, has not been reported previously from alfalfa roots in Canada. It has been isolated from a small percentage of diseased alfalfa roots in Wisconsin and California (12, 18).

*Rhizoctonia solani* has been isolated frequently from roots in Manitoba (7) and in many other regions of North America (12, 16, 23).

*Plenodomus meliloti* Mark-Let. (= *P. meliloti* D. & S.) is a common isolate from diseased alfalfa roots in Alberta and Saskatchewan (22) but has not been reported previously from Manitoba.

The remainder of the identified isolates are usually regarded as common soil inhabitants and include members of the following genera: *Alternaria*, *Coprinus*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Dendryphium*, *Gliocladium*, *Monotospora*, *Mucor*, *Myrothecium*, *Papularia*, *Penicillium*, *Periconia*, *Phoma*, *Pullularia*, *Stemphylium*, *Stysanus*, *Torula*, *Trichoderma*, *Trichothecium*, *Tubercularia*, and *Volutella*.

#### *Distribution in Relation to Soil Type*

The effect of soil type on the relative prevalence of the predominant fungi is shown in Table 1. Very distinct distributional patterns were obtained for several of the fungi listed. The predominance of *Pyrenochaeta*

*terrestris* in sandy soils is in agreement with Sprague's report of its prevalence in dry, sandy soils of North Dakota (24). *F. oxysporum* var. *redolens*, *F. oxysporum* and *F. solani* were isolated much more frequently in the heavy, black, highly organic type of soil than in either the sandy or high lime types. Bisby *et al.* (1) also found the *Fusaria* as a group to be more numerous in virgin meadow-prairie soils of the Red River valley than in soils from other regions in Manitoba. The relatively even distribution of *F. acuminatum* in all three soil types is an exception to this general trend.

The infrequent appearance of *Cephalosporium* sp. in isolations from sandy soils is in contrast to the conclusion of Bisby *et al.* (1) that members of this genus are rather evenly distributed in all soils of Manitoba. The distribution of *S. meliloti* on roots follows closely the observations made during the past years on the relative prevalence of Stagonospora leaf spot in Manitoba. The remainder of the fungi listed show comparatively little variation in percentage of total isolates from one soil type to another.

### Seasonal Distribution

The most striking example of the effect of season on the prevalence of the associated fungi is provided by *P. meliloti*. As shown in Table 2,

TABLE 2.—RELATIVE PREVALENCE OF THE FUNGI ISOLATED FROM ALFALFA ROOTS IN MANITOBA AT DIFFERENT TIMES OF THE YEAR

Fungus	Percentage of total isolates <sup>1</sup>		
	Spring	Summer	Autumn
<i>Pyrenochaeta terrestris</i>	13	20	24
<i>Fusarium acuminatum</i>	11	14	7
<i>Ascochyta imperfecta</i>	6	4	4
<i>F. oxysporum</i> var. <i>redolens</i>	1	7	4
<i>F. oxysporum</i>	Trace	6	5
<i>Cephalosporium</i> sp.	2	5	7
<i>F. solani</i>	0	4	3
<i>Cylindrocarpon ehrenbergi</i>	5	4	8
<i>Stagonospora meliloti</i>	1	1	3
<i>Rhizoctonia solani</i>	0	4	3
<i>Plendomus meliloti</i>	30	2	3
Miscellany identified	6	14	9
Miscellany unidentified	25	15	20
Total	100	100	100
Total number of isolates	342	1948	334

<sup>1</sup> Averages for the isolates obtained from the crowns and primary roots of second- and third-year alfalfa plants grown at three locations.

this fungus formed 30 per cent of the isolates obtained in the spring and only 2 and 3 per cent of those obtained in the summer and autumn respectively. This seasonal distribution can be correlated with the optimum temperature for growth and pathogenicity. The optimum temperature for growth of the Manitoba isolates was 16° C.; this temperature is the same as that found by Sanford (22) using Alberta isolates. Pathogenicity studies (22) have shown that this organism is only able to incite disease on alfalfa plants emerging from dormancy in the spring.

*F. acuminatum* was isolated in fairly equal numbers in both the spring and summer, but less frequently in the autumn. Cormack (9) reported this species to be slightly pathogenic in both winter and summer tests carried out in Alberta. *Ascochyta imperfecta* was slightly more prevalent in the spring. Cormack (11) reported that cool, moist soil favoured seedling disease, but that root lesions developed more slowly in the spring than in the summer tests with this organism. Observation of greater prevalence of *C. ehrenbergi* in the spring and autumn is consistent with Cormack's report of its virulence as a spring pathogen in Alberta (8).

*Stagonospora meliloti* was more prevalent in the fall than earlier in the season. Jones and Weimer (18) and Erwin (12) reported that root rot progresses slowly in plants inoculated with this organism; this slow development may account for the greater number of isolates obtained at the end of the season. *R. solani* was isolated in the summer and fall, but not in the spring. Manitoba isolates of this fungus grew best in culture at 24°-30° C. and as this temperature has been reported to be optimum for disease development on alfalfa (23), its prevalence in summer and not in the spring is thus explained. *F. solani* showed the same distributional pattern as *R. solani*, and for growth *F. solani* also has a relatively high optimum temperature of 30° C.

Of those fungi which have not been reported pathogenic to alfalfa, *P. terrestris* and *Cephalosporium* sp. showed increases in percentage of total isolates from spring to autumn. *F. oxysporum* var. *redolens* and *F. oxysporum* were isolated infrequently during the spring but were prevalent in the summer and fall.

### *Influence of Plant Maturity on Distribution*

The prevalence of the fungi isolated from alfalfa plants of various ages is indicated in Table 3. Isolations from one-month-old seedlings yielded predominantly *R. solani*, *P. debaryanum*, and the *F. oxysporum* group. In addition to the isolates obtained from these seedlings, all the 447 colonies obtained from seedlings which damped off within 2 days after emergence were identified as *P. debaryanum*. The first 2 fungi are recognized seedling pathogens of many crops but the importance of the latter is not so well defined. Mead\* isolated *F. oxysporum* var. *redolens* consistently from diseased sweet clover seedlings in Saskatchewan, but could not prove its pathogenicity.

The *F. oxysporum* group also predominated in the isolates obtained from first year plants, but gradually declined in importance as the plants became older. In contrast, *F. acuminatum* was isolated infrequently from

\* In Connors, I. L., and D. B. O. Savile, compilers. 29th annual report of the Canadian plant disease survey, 1949, p. 23. 1950.



TABLE 3.—INFLUENCE OF PLANT MATURITY ON THE RELATIVE PREVALENCE OF THE FUNGI ISOLATED FROM ALFALFA ROOT LESIONS

Fungus	Percentage of total isolates			
	1-month-old seedlings <sup>1</sup>	First-year plants <sup>2</sup>	Second-year plants <sup>3</sup>	Third-year plants <sup>3</sup>
<i>Pyrenochaeta terrestris</i>	1	1	17	20
<i>Fusarium acuminatum</i>	1	14	17	12
<i>Ascochyta imperfecta</i>	0	2	6	7
<i>F. oxysporum</i> var. <i>redolens</i>	18	27	8	4
<i>F. oxysporum</i>	17	22	5	3
<i>Cephalosporium</i> sp.	0	8	5	3
<i>F. solani</i>	8	2	4	4
<i>Cylindrocarpon ehrenbergi</i>	0	1	2	5
<i>Stagonospora meliloti</i>	0	0	Trace	6
<i>Rhizoctonia solani</i>	19	0	2	3
<i>Plenodomus meliloti</i>	0	0	2	1
<i>Pythium debaryanum</i>	17	0	0	0
Miscellany identified	15	12	18	14
Miscellany unidentified	4	11	14	18
Total	100	100	100	100
Total number of isolates	346	133	922	1446

<sup>1</sup> Averages of the isolates obtained from seedlings grown in soil from three locations.

<sup>2</sup> Averages of the isolates obtained during the summer of 1951 from the crown and roots of alfalfa grown at three locations.

<sup>3</sup> Averages of the isolates obtained during the summer from crown, vascular region, primary roots, and noncambial roots of alfalfa grown at three locations.

seedlings but increased to a dominant position among the isolates from older plants. Similar results were obtained in a survey of the fungi associated with red clover root rot in Wisconsin (19). *P. terrestris* was of minor importance on seedlings and first-year stands but was the most frequently isolated fungus obtained from second- and third-year plants.

*A. imperfecta* and *S. meliloti* did not appear among the isolates from seedlings, but assumed increasing importance on older stands. These pathogens have been isolated from seed (11) and, in the case of the former, have been shown to incite seedling damping-off (21). The seeds used in this survey, however, were surface sterilized in order to obtain an indication of the fungi in different soils rather than on seeds. With a different method of surveying, it is possible that these fungi might be found on seedlings in Manitoba. Both of these fungi are foliage pathogens and their prevalence on older plants can be traced to the yearly build-up of inoculum in the debris of foliage-infected plants.

*C. ehrenbergi* was not isolated from seedlings but increased in importance after the first year's growth. Cormack (8) reported that seedlings and young plants of alfalfa were less susceptible to attack by this fungus than older plants. *R. solani*, although prevalent on seedlings and second- and third-year plants, was not isolated from first-year plants. *P. meliloti* was not isolated from seedlings or first-year plants, but it was isolated following the first winter dormancy period. *F. solani* appeared in greater numbers from seedlings than from plants of greater maturity.

#### *Distribution in the Root System*

Data in Table 4 show the prevalence of the predominant fungi in relation to the part of the root system from which they were isolated. Most of the species were obtained in greater numbers from a specific part of the root system than from other parts. In most cases this specificity substantiated studies with these fungi by earlier workers.

*F. acuminatum* and *R. solani* were isolated more frequently from crown tissues than from other parts of the root system. Hawn and Cormack have reported these fungi as part of a crown bud rot complex in Alberta, *F. acuminatum* comprising 42 per cent of the isolates and *R. solani* 28 per cent (16).

TABLE 4.—RELATIVE PREVALENCE OF THE FUNGI ISOLATED FROM VARIOUS PARTS OF THE ROOT SYSTEM OF SECOND- AND THIRD- YEAR ALFALFA PLANTS

Fungus	Percentage of total isolates <sup>1</sup>			
	Crown	Vascular	Primary	Noncambial
<i>Pyrenochaeta terrestris</i>	16	9	23	25
<i>Fusarium acuminatum</i>	23	24	6	5
<i>Ascochyta imperfecta</i>	4	15	4	3
<i>F. oxysporum</i> var. <i>redolens</i>	8	2	6	8
<i>F. oxysporum</i>	7	3	4	2
<i>Cephalosporium</i> sp.	8	6	3	0
<i>F. solani</i>	3	6	5	3
<i>Cylindrocarpon ehrenbergi</i>	2	3	6	3
<i>Stagonospora meliloti</i>	1	10	2	0
<i>Rhizoctonia solani</i>	6	1	3	1
<i>Plenodomus meliloti</i>	Trace	0	3	3
Miscellany identified	12	14	15	20
Miscellany unidentified	8	7	20	27
Total	100	100	100	100
Total number of isolates	641	140	1307	280

<sup>1</sup> Averages for the isolates obtained during the summer from alfalfa grown at three locations.



FIGURE 1. Relative pathogenicity of 7 species of fungi on alfalfa seedlings grown in sand at four temperatures for 14 days. Top to bottom: 16°, 20°, 24°, and 28°C. Left to right: control, *Pyrenochaeta terrestris*, *Fusarium oxysporum* var. *redolens*, *F. solani*, *F. acuminatum*, *F. oxysporum*, *Rhizoctonia solani*, and *Pythium debaryanum*.

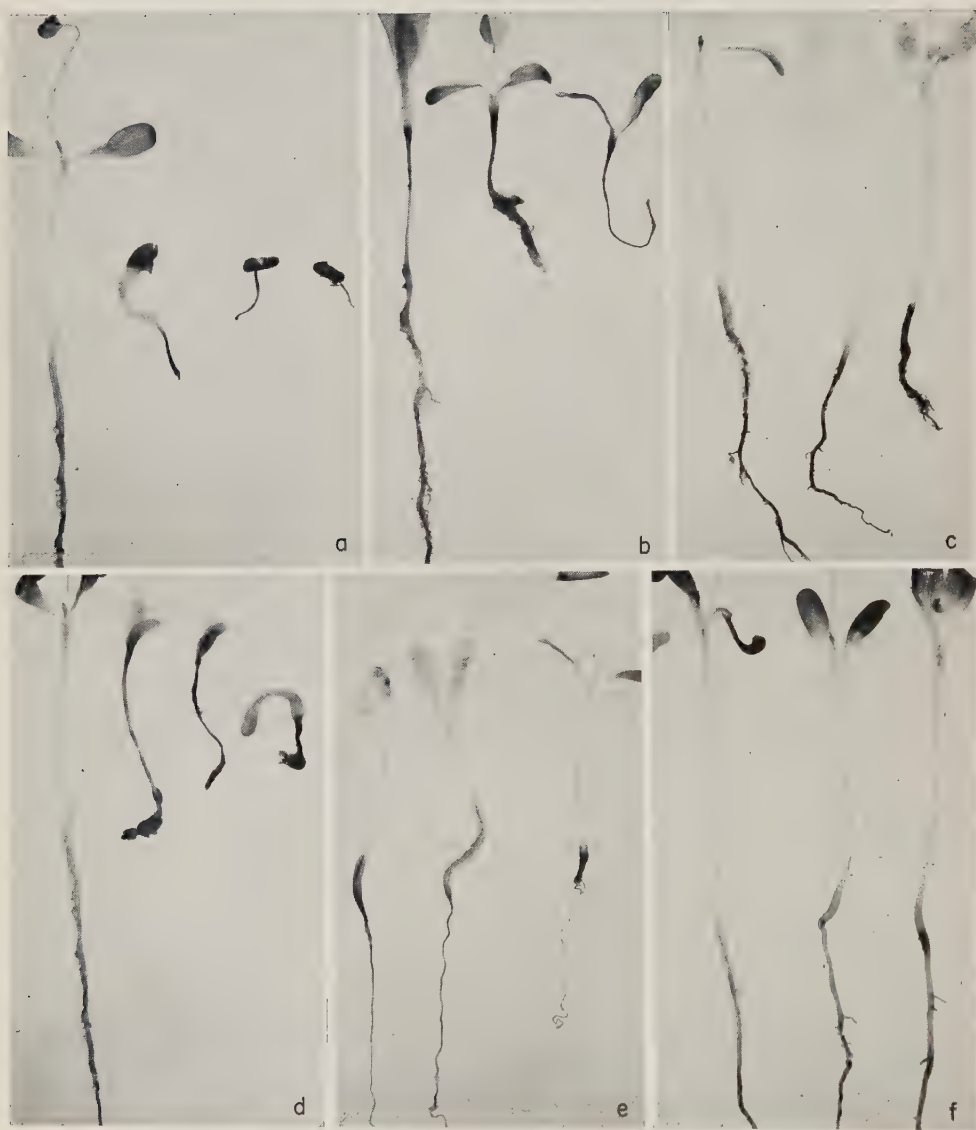


FIGURE 2. Typical symptoms produced on alfalfa seedlings grown in sand infested with the following organisms: (a) *Pythium debaryanum*, (b) *Rhizoctonia solani*, (c) *Pyrenochaeta terrestris*, (d) *Fusarium acuminatum*, (e) *F. solani*, and (f) *F. oxysporum* var. *redolens*. Plant on the left in (a) and (d) is not inoculated.



*A. imperfecta* and *S. meliloti* appeared to be definitely associated with discoloration of the interior of the root, both organisms being isolated much more frequently from that region. The latter fungus has been reported to cause a reddish flecking of the woody tissues of an infected crown branch or root (12, 18), but this type of symptom did not appear among those described by Cormack (11) for the root disease incited by *A. imperfecta*.

There was very little difference<sup>\*</sup> between the percentages of the various fungi obtained from the primary roots and the noncambial roots. *P. terrestris*, *C. ehrenbergi* and *P. meliloti* were isolated more frequently from these roots than from the crown and vascular tissues.

### *Pathogenicity Studies with the Associated Fungi*

The 7 species of fungi which predominated among those isolated from one-month-old seedlings were tested for their pathogenicity on alfalfa seedlings grown in sand at temperatures of 16°, 20°, 24°, and 28° C. The results are illustrated in Figure 1. *P. debaryanum* was the most pathogenic; it killed all seedlings at all four temperatures. Similar results have been reported by Halpin *et al.* (15). *R. solani* killed all seedlings at 28° C., but was less injurious at lower temperatures. The pathogenicity of strains of this fungus on alfalfa seedlings has been reported frequently (12). The majority of the seedlings inoculated with *F. acuminatum* and *F. oxysporum* failed to emerge within the 14-day period but many were alive. Disease severity was greatest at the higher temperatures on seedlings inoculated with *F. oxysporum*, but it was similar at all four temperatures on those inoculated with *F. acuminatum*. *F. solani* incited no pre-emergence damping-off but some post-emergence blighting occurred at 28° C. *F. oxysporum* var. *redolens* and *P. terrestris* were relatively non-pathogenic at all temperatures. Mead\* has reported the former to be also non-pathogenic to sweet clover seedlings.

The various fungi produced characteristic symptoms on inoculated alfalfa seedlings. *P. debaryanum* incited pre-emergence damping-off, very few seedlings emerging at any temperature. The appearance of the infected seedlings, as illustrated in Figure 2*a*, corresponded to the description given by Buchholtz (4). *F. acuminatum* incited symptoms markedly different from those of Pythium damping-off. The majority of the seedlings were not killed but were badly stunted and malformed and for this reason it was difficult to evaluate the emergence in the stand. The hypocotyl of many plants was swollen and had not elongated (Figure 2*d*). In most cases the root and lower portion of the hypocotyl were rotted off. The cotyledons were distinctly darker in colour than those of the check plants. Seedlings inoculated with *F. oxysporum* were also difficult to evaluate in terms of stand as the majority were badly stunted and although alive had not emerged. The hypocotyls and roots of many seedlings were twisted and misshapen with the lower part of the root completely rotted off. *R. solani* incited both pre- and post-emergence damping-off at temperatures higher than 16° C. A typical dark brown to black collar rot, as described by Bruehl (3) and illustrated in Figure 2*b*, was observed.

\* See footnote page 314.

*F. solani* incited symptoms similar to those of *Fusarium* root rot of pea. Longitudinal, reddish-brown lesions appeared on the hypocotyls and roots. The lower tap root was completely rotted off in many cases but very few plants were killed within the 14 days (Figure 2e). *P. terrestris*, although causing no damping-off or decrease in vigour of inoculated seedlings, did infect the roots. The roots were distinctly brown in appearance and pycnidia of the fungus were abundant (Figure 2c). Kreutzer reported that no symptoms of pink root were produced on alfalfa seedlings inoculated with this fungus (20). No distinct type of injury was apparent on seedlings inoculated with *F. oxysporum* var. *redolens* (Figure 2f).

The results of pathogenicity studies on 3-month-old plants largely confirmed those obtained by previous workers. The crown buds of plants inoculated with *R. solani* were severely infected particularly at the higher temperatures, and appeared blackened and shrivelled. The fungus was always reisolated from surface-sterilized diseased buds. The majority of the roots were completely healthy. Similar symptoms of crown and bud rot without root lesioning have been reported by Erwin in California (12) and Hawn and Cormack in Alberta (16).

A crown bud rot was evident also on plants inoculated with *A. imperfecta* but in this case the disease was most severe at the lower temperatures. Numerous lesions on the roots and crowns occurred at 16° and 20° C. and a few were evident at the higher temperatures. Rotting of the crown buds of alfalfa by this fungus has been reported by Johnson and Valleau (17) who believed the disease was a major cause of gradual reductions in stands of this crop in Kentucky. Cormack, however, regards *A. imperfecta* as of less importance in Alberta than the other root pathogens reported from that province (11). This latter conclusion was based on inoculation studies with wounded and unwounded tap roots, and did not take into consideration the damage resulting from crown bud infection.

Stagonospora crown and root rot, as described by Jones and Weimer (18), was produced on plants inoculated with *S. meliloti*. Typical symptoms included a brown lesion with roughened surface below the crown branches and a reddish flecking of the vascular tissues.

The results obtained with plants inoculated with *P. terrestris*, *F. oxysporum* var. *redolens*, *F. acuminatum* and *F. oxysporum* were inconclusive because no definite type of injury appeared to be associated with any of the fungi. A considerable amount of rotting developed at the base of branch roots of plants inoculated with each of the fungi but this type of injury also occurred on some uninoculated check plants particularly at the highest temperature. Reisolations from such lesions yielded various species of *Fusarium*, predominantly *F. oxysporum* var. *redolens*. Cormack reported *F. acuminatum* to be weakly pathogenic to field grown alfalfa roots in both winter and summer tests (9).

Three of the fungi used in the test, *C. ehrenbergi*, *Cephalosporium* sp. and *F. solani* proved to be non-pathogenic under the conditions imposed. *C. ehrenbergi* has been reported by Cormack (8) to be pathogenic to alfalfa under certain experimental conditions. In his experiments, summer and fall inoculations were made on roots growing in the field by applying inoculum directly to the tap roots. He found that disease severity was

greatest in the spring following fall inoculation and that the roots of plants 3 and 4 years old were more severely attacked than those of younger plants. None of these conditions was met in the present test. Conflicting conclusions as to the pathogenicity of *F. solani* have been reported. Erwin (12) was not able to produce crown or root rot on alfalfa plants grown in sterilized soil infested with the organism, but Staten and Leyendecker (25) reported that between 40 and 80 per cent of the plants surviving after 18 weeks from date of inoculation showed stunting and leaf chlorosis. In the latter work, inoculations were made by planting seed in infested soil and by introducing the fungus into the split crowns of healthy transplants.

### DISCUSSION

This investigation has shown that more than one fungus must be considered in determining the factors responsible for root and crown rotting of alfalfa in Manitoba. Hitherto, in this province, *Rhizoctonia solani* has been regarded as the only important incitant of root and crown rot, particularly the latter (7). Although this species did appear among the more predominant fungi isolated, a number of other fungi reported to be pathogenic to alfalfa in other regions were equally or more prevalent. *A. imperfecta*, *C. ehrenbergi*, *S. meliloti*, and *P. meliloti* all have been proved pathogenic in areas having the same general climatic conditions as exist in Manitoba, but never have been associated previously with the disease in this province. In addition, a number of fungi were isolated which are usually regarded as saprophytes or weak pathogens. In this group *P. terrestris*, *F. acuminatum*, *F. oxysporum* var. *redolens*, *F. oxysporum*, *Cephalosporium* sp., and *F. solani* predominated.

The complex of fungi contributing to the deterioration of alfalfa stands in Manitoba is similar to that occurring in Alberta. The members of this complex affect the plant at various stages in its growth and are most important under specific environmental conditions. Damping-off of seedlings in cool spring weather can be attributed mainly to infection by *P. debaryanum* and to a lesser extent by *F. oxysporum*, *F. acuminatum*, and probably *A. imperfecta*. If the seeds are sown later in the spring during warm weather *R. solani* may cause a decrease in stand that is additional to the decrease resulting from infection by the other four organisms. Once the seedlings have become well established, *P. debaryanum* and *R. solani*, judging from their absence in isolations from first-year plants, are of no importance.

Species of *Fusarium* predominated among the fungi obtained from first-year plants. It is believed, however, that they are of little importance in causing rot development during the first year as they were relatively non-pathogenic in the tests on mature plants and disease severity was very low on field-grown plants the first year.

In the spring of the second year, and each year thereafter, *P. meliloti* and *C. ehrenbergi* are, according to Sanford (22) and Cormack (8), capable of inciting disease on plants emerging from winter dormancy. Although pathogenicity tests made during this investigation were not successful, the prevalence of these fungi in spring isolations demonstrates the occurrence in Manitoba of the diseases they incite. The results of isolations in the



spring and pathogenicity tests at low temperature indicated that *A. imperfecta* also is able to incite a rot, particularly of the crown buds, at this time of year. *F. acuminatum* was prevalent in the spring and was usually associated with crown lesions. This distribution might indicate that, as this species was relatively non-pathogenic when inoculated on healthy plants, it contributes to the decay initiated by these other fungi. The low-temperature basidiomycete, causing winter crown rot of alfalfa in Alberta (10), was not isolated from the areas covered in this survey. It has been isolated from more northerly regions of Manitoba, however, and there it must be considered a part of the complex.

Another group of fungi, which have a higher optimum temperature for growth than those mentioned above, appears more often in isolations from diseased plants as the season progresses into the warmer periods of summer and fall. This group included *F. acuminatum*, *F. oxysporum*, *F. oxysporum* var. *redolens*, *F. solani*, *P. terrestris* and *R. solani*. Of these, the last species is the most important on the basis of pathogenicity tests made on mature plants. The other species are believed to be saprophytes which occur in root lesions caused earlier in the season, or weak pathogens of plants predisposed to infection by wounding, excessive grazing or cutting, or unfavourable environmental conditions such as severe summer drought.

Stagonospora root and crown rot has not been reported previously in Canada. As a result of the pathogenicity tests with mature plants, it is believed that Manitoba isolates of *S. meliloti* are capable of inciting a severe disease on alfalfa. The distribution of the disease in the province is apparently dependent on the amount of foliage disease occurring in the particular region. The incidence of leaf spotting is low in the black soil region because alfalfa is cut for hay before leaf lesions become numerous and in this area *S. meliloti* is infrequently isolated from roots. In contrast, where alfalfa is grown as a seed crop in the high lime and sandy soils lesions on the leaves develop throughout the summer. It is from those areas that the majority of isolates of the fungus were obtained from roots.

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